ISSN 2790-086X
DOI:10.6156/JTAR

Vol. 75

台灣農業研究

Journal of Taiwan Agricultural Research • No.1 2026

台灣農業研究

Journal of Taiwan Agricultural Research

Vol.75 No.1 2026

出版者
農業部農業試驗所

Published by
Taiwan Agricultural Research Institute,
Ministry of Agriculture

ISSN 2790-086X

台 灣 農 業 研 究

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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):1–26 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0001

Feature Article

Integrated Pest Management Strategies for Strawberry Production in Taiwan: A Review of Technological Innovations and Implementation Challenges

Pei-Che Chung[1,*], Qiao-Juan Lai[2], Jen-Hao Yeh[3], Jeng-Hsien Tsai[4], Yi-Pei Li[5], Che-Hao Cheng[3], and Hsiu-Ying Lu[6]

Abstract

Chung, P. C., Q. J. Lai, J. H. Yeh, J. H. Tsai, Y. P. Li, C. H. Cheng, and H. Y. Lu. 2026. Integrated pest management strategies for strawberry production in Taiwan: A review of technological innovations and implementation challenges. J. Taiwan Agric. Res. 75(1):1–26.

Strawberry is a highly valuable economic crop with a domestic cultivation area of approximately 586 ha, primarily concentrated in Miaoli County. During the strawberry production season, a large number of tourists are attracted, which drives the development of the surrounding industries. Over the past decade, the strawberry industry has faced increasing challenges, including threats from diseases and pests, varietal transitions, and climate change, making cultivation progressively more difficult. Based on the principle of integrated pest management (IPM), which emphasizes prevention over treatment, our research team has implemented the following research and extension strategies tailored to different cultivation stages: (1) Establish a strawberry healthy runner plant supply chain by developing diagnostic techniques for key pathogens and integrating these into the voluntary pathogen-free certification system for strawberry propagation, while guiding the industry toward specialized nursery production and propagation; (2) Develope a screening platform to assess the resistance and susceptibility of domestic strawberry germplasm to manage major diseases, by integrating molecular marker-assisted breeding and high-throughput phenotyping technologies to reduce the reliance on extensive field selection and accelerate the breeding process; (3) Adapt nursery propagation and fruit production management strategies to mitigate the impact of high temperatures on strawberry production due to climate change; (4) Provide technical guidance to farmers on pre-planting soil disinfestation, optimizing base fertilizer according to soil analysis data, and implementing rationalized liquid fertilizer application after transplanting; (5) Collaborate with interdisciplinary teams to develop machine learning and deep learning algorithms for the automated identification of strawberry diseases and pests while integrating an expert decision support system to enable real-time and accurate diagnosis and management recommendations for farmers. Through the promotion of IPM strategies, strawberry yield and quality will be gradually improved, increasing

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Received: March 11, 2025; Accepted: October 29, 2025.
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farmer profitability and creating an environmentally friendly approach while meeting consumer market demand.

Key words: Healthy runner plants, Disease-resistant breeding, Climate change, Soil disinfestation, Deep learning.

INTRODUCTION

Strawberry (*Fragaria ×ananassa*) is highly favored by consumers due to excellent taste, attractive appearance, and rich nutritional value. The domestic strawberry cultivation area in Taiwan is approximately 586 ha, with Miaoli County accounting for 90%, making it the most important production area. After integrating with agritourism, it has become a highly representative sixth-level industry. However, according to the annual pesticide residue monitoring reports on fruit and agricultural products published by the Ministry of Agriculture (MOA), strawberry has ranked first in pesticide residue detection rates for consecutive years, making this issue a major consumer concern. To enhance food safety in agricultural products, MOA has been actively promoting a policy to halve chemical pesticide risks within ten years, with integrated pest management (IPM) as the core promotional strategy. IPM refers to a comprehensive approach to managing crop pests by integrating multiple control methods to address single or multiple pest issues. Chemical pesticides are used only when necessary, emphasizing a prevention-first approach with chemical control as a supplementary measure (Stenberg 2017).

Approximately 29.25 million strawberry runner plants are required annually, with the propagation period occurring from April to September. However, the greatest challenge during the runner plant propagation period is disease management, particularly the occurrence of anthracnose rot during the high-temperature and rainy months of July and August. Since 2010, the industry has faced severe runner plants shortages due to anthracnose rot (Chung *et al*. 2020). Most of the current runner plant propagation methods still rely on farmers cultivating strawberry runner plants in open-field environments with overhead irrigation, and it is also unknown whether the mother plants are infected with the pathogen, resulting in serious latent infections of anthracnose rot. Starting in 2018, the main strawberry cultivar in the production area gradually shifted from ‘Taoyuan No. 1’ to ‘Xiang-Shui’, and by 2019, the planting area of ‘Xiang-Shui’ rapidly increased to approximately 80%. Because ‘Xiang-Shui’ is highly susceptible to leaf blight, this varietal transition has caused leaf blight to emerge as a significant disease (Chung *et al*. 2021; Wu *et al*. 2021). Anthracnose rot has the characteristic of latent infection (Prusky 1996), while leaf blight has not yet been confirmed to be capable of latent infection. However, both diseases can be transmitted through asymptomatic runner plants or mother plants with rainfall and irrigation water contributing to the splash dispersal of conidia (Chung *et al*. 2022; Alam *et al*. 2024). The high temperature and intermittent heavy rains during the monsoon and typhoon seasons create environmental conditions conducive to infectious disease and epidemics, causing farmers to apply fungicides more frequently. If infected plants are not thoroughly removed during the runner plants propagation period, large-scale disease outbreaks often occur after transplanting, ultimately leading to wilting and plant death. In 2015, after strawberry transplanting, several fields in Dahu Township and Shitan Township experienced a replanting rate of up to 30%. In addition to anthracnose rot caused by pathogen-infected runner plants, the high incidence of disease in some fields may have resulted from the accumulation of *Fusarium oxysporum* f. sp. *fragariae* (Chung & Wu 2017). According to estimates from the Dahu Area Farmers’ Association in 2023, the yield reduction due to Fusarium wilt in the entire township was approximately 10–20%. Similar situations were observed in neighboring strawberry production

areas. On-site field assessments revealed that the causes of Fusarium wilt could be attributed to several factors, including pathogens that were latently infected in the runner plants or residues in the soil, early planting (from mid-September to early October) during high temperatures, improper water management after transplanting, and excessive fertilization. The damage rate of Fusarium wilt ranged from 30–90%, and compared to 2015, there was an additional factor of varietal change, from 'Taoyuan No. 1' to 'Xiang-Shui' (unpublished data).

In order to address the issue of frequent fungicide usage during strawberry runner plants propagation (Chung *et al*. 2022), and to reduce the usage of chemical pesticides during fruit production, our research team has implemented an IPM strategy, including the establishment of disease identification techniques for rapid confirmation of pathogen species, allowing for the development of targeted control strategies to minimize unnecessary fungicide usage by farmers. We have also guided farmers to gradually change their propagation methods, promoting a healthy runner plant propagation system; adaptive cultivation management strategies have been implemented to mitigate the impact of adverse conditions of climate change on strawberry, such as heat stress during the propagation period or early transplanting stages, which could lead to heat-related disorders or accelerated Fusarium wilt. Soil disinfestation and rationalized fertilization are conducted in the fields before runner plant transplanting, while natural enemies are released at appropriate timing during the fruiting period to control small pests. Our IPM strategy involves guiding farmers to reduce the frequency and amount of chemical pesticide usage, while ensuring economic benefits for farmers and promoting environmental sustainability, to produce high-quality and safe strawberry (Birch *et al*. 2011; Dara 2019; Huang *et al*. 2022; Angon *et al*. 2023). This review focuses on the current status of the strawberry industry in Taiwan, addressing field issues by applying established or developing IPM strategy. It also compiles relevant IPM technology from major strawberry-producing countries to inform future research and practical implementation.

STRAWBERRY IPM IMPLEMENTATION STRATEGY

Disease challenges in subtropical strawberry systems

The proportion of propagated strawberry runner plants by farmers remains above 70%, relying mostly on asexual propagation from mother plants retained in fruiting fields and cultivated in open-field environments in Taiwan. Anthracnose rot, leaf blight, Fusarium wilt, and angular leaf spot are the primary diseases causing large-scale runner plants wilt and mortality during the propagation stage or resulting in replanting rates exceeding 50% after transplanting. Therefore, the foundation of strawberry IPM must begin with healthy runner plants.

Anthracnose rot

Anthracnose rot is one of the most devastating diseases in the strawberry industry, causing severe economic losses. Initially, infected leaves develop dark brown spots that gradually expand; lesions on petioles and runners become sunken, and salmon to red conidial masses could be observed under high humidity. When the crown is infected, the aerial parts of the plant exhibit wilting symptoms, often leading to replanting rates exceeding 30–40% after transplanting (Chung *et al*. 2019). Multilocus sequence analysis coupled with morphological characterization identified five *Colletotrichum* species associated with strawberry anthracnose rot in Taiwan, including *Colletotrichum siamense* and *C. fructicola* (*C. gloeosporioides* species complex), *C. boninense* and *C. karstii* (*C. boninense* species complex), and a newly identified species, *C. miaoliense* (*C. acutatum* species complex) (Chung *et al*. 2020). Additionally, *C. siamense* is the predominant species isolated from major strawberry production areas in Taiwan. Mycelial

growth rate assessment at different temperatures indicates that its optimal growth temperature is approximately 27.9℃, in accordance with the observation that strawberry anthracnose rot is prevalent under high temperature and humid conditions in Taiwan. However, after transplanting in the fields, as the temperature significantly drops in December, the incidence of anthracnose rot also declines accordingly. Due to the common practice of open-field sprinkler irrigation for runner plants propagation and the lack of healthy mother plants, the incidence of latent infection by *C. siamense* has become increasingly severe.

Leaf blight

Phylogenetic analysis confirmed *Neopestalotiopsis rosae* as the primary pathogen causing strawberry leaf blight (Wu *et al*. 2021). In the field, the pathogen primarily infects leaves, crowns, petioles, runners, fruits, and roots of strawberry. Symptoms on leaves appear as nearly circular, elliptical, or elongated brown lesions with concentric rings, and black conidial masses are visible in the lesion center. When the crown is infected, affected leaves develop reddish-brown to purplish-brown interveinal discoloration covering one-third to two-thirds of the leaf area. Occasionally, small light brown to white spots resembling sunburn symptoms appear, and on the abaxial surface, net-like sunken brown lesions can be observed. Infected plants exhibit dwarfing, poor development of new leaves, reduced runner production, and decreased flowering and fruiting. Under severe infections, extensive leaf wilting and plant death may occur (Lai & Wu 2024). As the dominant cultivar has shifted from 'Taoyuan No. 1' to 'Xiang-Shui,' the incidence of leaf blight has significantly increased.

Fusarium wilt

Fusarium oxysporum f. sp. *fragariae*, the pathogen of Fusarium wilt, invades the plant and causes vascular occlusion, leading to functional loss and asymmetrical growth of the trifoliate leaves (Chung & Wu 2017; Pastrana *et al*. 2019). The development of malformed leaves often results in one-sided wilting, loss of leaf luster, crown and root discoloration, and root rot. Infected plants gradually weaken, shrink in size, and eventually wilt, making this a relatively slow-progressing disease. *F. oxysporum* f. sp. *fragariae* is a strawberry-specific forma species infecting only strawberries and not other crops such as cucumber, tomato, or eggplant. The disease can develop between 18–25℃, with an optimal growth temperature for the pathogen at 28℃, and higher temperatures accelerate disease progression (Chen *et al*. 2017). Additionally, this disease can persist in the soil for several years through chlamydospores, making it necessary to remove infected plant residues and conduct soil disinfestation to prevent recurring outbreaks.

Angular leaf spot

The primary pathogen causing strawberry angular leaf spot has been identified as *Xanthomonas fragariae* through phylogenetic analysis (Wu *et al*. 2020). The disease is spread via dew, rain, or overhead irrigation. High environmental humidity or the presence of leaf wounds increases the likelihood of infection. Infected leaves eventually wilt and remain in the soil or substrate, potentially serving as the initial inoculum source for the following season. Field observations indicate that the pathogen can currently infect most cultivated strawberry cultivars. *X. fragariae* can infect leaves, petioles, crowns, roots, sepals, and fruits, with the most prominent symptoms observed on leaves. The disease initially appears as water-soaked angular lesions restricted by leaf veins on the abaxial surface. When viewed under light or transmitted light, the lesions appear translucent. As the disease progresses, lesions turn from translucent to reddish-brown, sometimes with yellow margins, and in severe cases, lesions expand and coalesce, leading to complete leaf necrosis. Under high humidity, milky white to pale yellow bacterial exudates may form on the lesion surface. When sepals are infected, water-soaked angular symptoms also develop. Infected fruits exhibit yellowish and hardened flesh near the

attachment point to the sepals, rendering them unmarketable. An appropriate preventive strategy is to avoid planting runner plants infected with this disease.

Technological advances in IPM

Establishing a strawberry healthy runner plant supply chain

Use of healthy runner plants is the essential first step in strawberry IPM. To ensure that propagated runner plants are free from specific pathogens, countries such as the United States and Japan have established disease inspection standards and certification programs. In response to the demand for healthy runner plants, relevant regulations have been implemented, and the supply chain for healthy runner plants is being developed through collaboration between industry, government, academia, and research institutions in Taiwan.

1. Progress and applications of healthy runner plants propagation in Taiwan and other countries

California has the largest strawberry production area in the United States, and with a well-developed healthy strawberry runner plant propagation system that local farmers recognize for its benefits. In California, strawberry cultivars are developed by the Department of Plant Sciences at the Davis campus of the University of California (UC Davis), patented and licensed by UC Davis Technology Transfer Services, and maintained and distributed by Foundation Plant Services (FPS) (http://fps.ucdavis.edu). Additionally, the FPS is responsible for maintaining and distributing these cultivars. The nursery system consists of Foundation plants, Registered plants, and Certified plants. Foundation plants are the initial, pathogen-tested, true-to-type stock, from which Registered plants are clonally propagated under controlled conditions. Registered plants serve as the source material for mass-produced Certified plants intended for commercial fruit production. Throughout the process, strict health and varietal purity standards are maintained. Furthermore, the sale of runner plants requires pre-ordering in the previous year to facilitate nursery propagation planning.

Tochigi Prefecture is the largest strawberry producing area in Japan. It has more than 600 ha of strawberry cultivation, and the strawberry healthy runner plants propagation system has been implemented for decades. At present, 100% of farmers used the runners propagated following the healthy runner plants supply system. Most of the new cultivars released from Tochigi Prefecture are virus-free through tissue culture to obtain the nuclear stock. The plants of the nuclear stock in the provenance greenhouse are virus-free, and then the original stock is propagated from the nuclear stock. Plants propagated from the original stock are moved to the original nursery for cultivation, and these are the mother plants subsequently provided to farmers. Runners propagated from the mother plants in the nurseries are used for transplanting into the field (Ishikawa 2011).

To prevent the spread of diseases and improve the health of runner plants produced by strawberry nurseries, the MOA in Taiwan has established a voluntary pathogen-free certification system for strawberry propagation in 2018 (Chung *et al*. 2022). The propagation of strawberry runner plants is divided into several stages: G0, which refers to tissue culture plantlets; G1, which is tissue culture plants that have been acclimatized in a controlled facility environment; G2, which is progeny plants derived from G1 plants, also propagated in regulated conditions and used for further propagation; and G3, which is G2 plants propagated under regulated conditions and used for transplanting into the production field. The environmental conditions and management practices required for the propagation of each stage are based on established guidelines, including elevated beds 40 cm above the ground, specific mesh sizes for insect nets at each stage, and the use of sterilized cultivation media and water sources. Notably, to maintain the quality

of runner plants, it is crucial that no pathogens such as anthracnose rot (*Colletotrichum* spp.), Fusarium wilt (*Fusarium oxysporum* f. sp. *fragariae*), Strawberry mild yellow edge virus, or root lesion nematodes (*Pratylenchus* spp.) can be detected at any stage. Verified cultivars of runner plants are propagated, including ‘Taoyuan No. 1’, ‘Xiang-Shui’, ‘Tainung No.1’, ‘Changzhi No.1’, ‘Changzhi No. 2’, and ‘Yuantu No.1’ in 2024.

For the demand of healthy runner plants, in addition to certified runner plants propagated following the voluntary pathogen-free certification system, non-certified high-quality runner plants propagated from tissue culture mother plants have also been introduced as an initial production type by nurseries. These non-certified plants will be gradually incorporated into the certification system based on production capacity. For the main cultivar ‘Xiang-Shui’ in major production areas, a total of 38,440 G1-stage runner plants were produced by nurseries in 2024, of which approximately 8,040 were certified plants. These certified plants were primarily purchased by farmers for use as mother plants to propagate additional runner plants. At the G3 stage, 215,000 runner plants were propagated and directly supplied to farmers for field planting.

The complete three-stage propagation system has not yet been fully implemented in Taiwan, primarily due to several factors, including the delayed production of G0 plantlets, the high labor demand during the G1 production process, and the limited space, which requires waiting for seedlings of other crops to be cleared before acclimatization can take place. These constraints result in delayed G1 availability, subsequently compressing the time for G2 and G3 runner propagation. In recent years, adverse climatic conditions and disease outbreaks have significantly impacted strawberry production. Several nursery operators have expressed interest in propagating healthy strawberry runner plants, leveraging their existing tissue culture propagation technology, along with well-equipped facilities such as greenhouses, soil and water sterilization systems. As a result, the quantities of healthy strawberry runner plants are expected to increase annually.

2. Development of detection methods for anthracnose rot and Fusarium wilt

To establish a propagation and supply chain for healthy strawberry runner plants, key diseases such as anthracnose rot and Fusarium wilt are detected at different propagation stages. During strawberry runner plants propagation, while mother plant is latently infected with anthracnose rot, the disease could spread to runner plants through rainwater or irrigation splash. The detection methods for anthracnose rot latent infection could be categorized into two major types: culture-based methods and polymerase chain reaction (PCR)-based molecular detection methods. Culture-based methods include the Paraquat method, the freezing method, and the SDEI (simple diagnosis by ethanol immersion) method (Cerkauskas 1988; Ishikawa 2003; Mertely & Legard 2004). The advantages of these methods are their simplicity and low cost, making them easier for farmers or nursery operators to implement. However, these methods require a longer time (typically 3–10 d) to obtain results and rely on spore morphology for pathogen identification. In contrast, the molecular detection methods offer high specificity and low detection limits, facilitating internal quality control for nurseries while significantly reducing the time required for detection compared to culture-based methods. The disadvantages include relatively high costs and the necessity for laboratory testing. Several molecular detection methods, including PCR, nested PCR, quantitative real-time PCR (qPCR), high-resolution melting analysis (HRM), and loop-mediated isothermal amplification (LAMP) (Freeman *et al*. 2001; Perez-Hernandez *et al*. 2008; Debode *et al*. 2009; Zhang *et al*. 2016; Rahman *et al*. 2019), have been applied for pathogen detection in strawberry. In recent years, we developed a nested PCR detection method capable of detecting the primary

pathogens *C. siamense* and *C. fructicola* (Chung *et al*. 2022), and it has been successfully applied to the voluntary pathogen-free certification system in Taiwan. Additionally, to facilitate on-site detection in fields or nurseries, a portable plant disease rapid screening kit has also been developed. Using a non-powered nucleic acid extraction kit, nucleic acids are extracted and then amplified via the LAMP method. Indicator dye (hydroxynaphthol blue, HNB) is added for visual interpretation of results, allowing on-site testing and result determination (Hung *et al*. 2024).

Infected strawberry mother plants with Fusarium wilt could transmit the pathogen to runner plants through the vascular bundles via stolons. To confirm that the mother plants used for propagation are free from this pathogen, selective culture medium FoG1 is employed for culturing (Nishimura 2007; Chung *et al*. 2018). If purple-red colonies appear, it is determined that the tested mother plant may carry the pathogen of Fusarium wilt and should not be used as a propagation source. Additionally, the application of multiplex PCR would assist in the screening of mother plants and the detection of residual soil pathogens (Suga *et al*. 2013). LAMP detection technology has also been developed in recent years and applied for screening strawberry germplasm preserved at Tochigi Prefecture Strawberry Research Institute in Japan (Katoh *et al*. 2021).

3. Development of detection methods for leaf blight and angular leaf spot

Molecular detection methods for strawberry leaf blight currently include HRM (Rebello *et al*. 2023) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Kaur *et al*. 2022). Primers targeting ITS, *TUB*, and *TEF* of strawberry leaf blight were designed in 2024 by our research team, and a nested PCR method was developed, with a detection limit of 100 fg of *Neopestalotiopsis rosae* DNA (Lai & Wu 2024).

The detection methods for strawberry angular leaf spot include enzyme-linked immunosorbent assay (ELISA), PCR, qPCR, and nested PCR. In recent years, Stöger & Ruppitsch (2004) developed a highly sensitive PCR kit called REDExtract-N-Ampk Plant PCR-Kit, which enhances PCR sensitivity by improving nucleic acid extraction efficiency, enabling the detection of *X. fragariae* on asymptomatic plants. Following this, real-time PCR was developed (Cubero *et al*. 2009), increasing sensitivity by approximately 100 times. Notably, Turechek *et al*. (2008) focused on detecting the strawberry crown, and LAMP further enhanced sensitivity and made it easier for farmers to use (Wang & Turechek 2016; Gétaz *et al*. 2017). Our research team is actively collaborating with academic research institutions to develop a detection method for angular leaf spot in Taiwan.

Currently, the disease diagnosis methods for strawberry runner plants include PCR testing for anthracnose rot and selective media for Fusarium wilt. Tissue-cultured mother plants are 100% tested, while tissue culture plantlets are randomly tested at a rate of 1/100, and acclimatized tissue-cultured plants are tested at a rate of 1/1000 when necessary. The verified tissue-cultured acclimatized plants, when introduced into the facility for propagation, result in a replanting rate of less than 1% after transplanting. In recent years, farmers have gradually adopted the concept of using healthy runner plants, leading to a growing demand for healthy strawberry runner plants. Some nurseries and biotechnology companies have also started to invest in this industry. However, after the acclimatized tissue-cultured plants are moved to the field, most farmers still propagate in open-field environments, and their management practices often lead to issues such as fertilizer burn and pesticide damage, making strawberry plants more susceptible to opportunistic pathogens such as *Fusarium* sp. from the environment, causing wilting. Therefore, future efforts will still need to focus on improving cultivation environments and management practices through education, training, and on-site guidance for farmers involved in propagation.

Disease-resistant breeding

Disease-resistant breeding is one of the fundamental methods for crop disease control and an essential strategy for implementing IPM. Taking strawberry leaf blight as an example, the 'Xiang-Shui' cultivar is highly susceptible, while 'Taoyuan No. 1' is disease-resistant. When formulating IPM strategies, it is necessary to consider the disease tolerance of different cultivar. Strawberry disease resistance breeding and research related to resistance genes have made significant breakthroughs with advancements in molecular genetics and genomics. For strawberry anthracnose rot, three resistance loci, namely *RCa2*, *FaRCg1*, and *FaRCa1*, have been identified (Anciro *et al*. 2018; Salinas *et al*. 2019). However, these loci were identified based on analyses using the foreign major pathogenic species *C. acutatum* and *C. gloeosporioides*, which differ from the main pathogenic species in Taiwan, *C. siamense* and *C. fructicola*. The trials showed that it was not applicable for predicting resistance or susceptibility to *C. siamense* and *C. fructicola*. This is presumably due to the species-specific nature of disease resistance genes (Miller-Butler *et al*. 2019). Therefore, further research is needed to identify detection genes for resistance to the major pathogens causing anthracnose rot in strawberry in Taiwan. Another important case is strawberry Fusarium wilt resistance breeding. In response to the impact of Fusarium wilt on the strawberry industry in California, the University of California conducted phenotypic screening and genotypic identification of resistance in local and international strawberry germplasms. By combining various molecular genetic tools, they discovered five Fusarium wilt resistance genes, *Fw1* to *Fw5*, and used molecular markers to assist in selecting and breeding multiple new Fusarium wilt-resistant cultivars suitable for different cultivation systems (Pincot *et al*. 2022).

In 2019, the RosBREED research project, funded by the United States Department of Agriculture and the Florida Strawberry Research and Education Foundation, released the strawberry DNA testing handbook (Oh *et al*. 2019). This handbook consolidates research information on DNA molecular markers for several important strawberry traits, including resistance genes for three major diseases: crown rot (*Phytophthora cactorum*), angular leaf spot (*X. fragariae*), and anthracnose rot (*C. acutatum*), along with their molecular marker sequences, testing conditions, result interpretation, and reference cultivars. We used these molecular markers to genotype the strawberry germplasm stored at our facility. Preliminary results indicated that the anthracnose rot resistance gene molecular marker *RCa2* was ineffective in screening for resistance against the major strains of the pathogen in Taiwan. Additionally, attempts to design T-ARMS PCR molecular markers to detect resistance genes *FaRXf1* for angular leaf spot and *FaRCg1* for anthracnose rot also did not yield precise and directly applicable results, so further testing is needed (Yeh & Wei 2024).

In Taiwan, phenotypic evaluation of strawberry disease resistance was conducted by Wu *et al*. (2022), who screened 55 domestically preserved strawberry germplasms for anthracnose rot resistance by inoculating them with the representative local isolates *C. siamense* ML133 and *C. fructicola* ML356. Five cultivars (lines) exhibited significantly lower disease severity compared to the susceptible control cultivar 'Taoyuan No.1.' Among them, 'Fukuba', 'Tufts', and 'Solana' showed significantly lower conidial germination rates and appressorium formation rates on leaf surfaces compared to 'Taoyuan No.1'. Additionally, no accumulation of hydrogen peroxide was observed in infected leaf tissues, indicating that the resistance carried by these three cultivars effectively delayed the infection of *C. siamense* and *C. fructicola* (Wu 2020). The preserved line 'TYS16109' at Taoyuan District Agricultural Research and Extension Station exhibited a disease incidence area of less than 10% in both pathogen inoculation tests, showing a significant difference from 'Taoyuan No.1' and demonstrating good resistance in both leaves and crowns, making it a promising candidate as a parent in future disease-resistant breeding programs. The emerging leaf blight of strawberry

in Taiwan is caused by *Neopestalotiopsis rosae*, and the currently dominant cultivar 'Xiang-Shui' lacks resistance to this pathogen. Through detached leaf inoculation assays of 44 preserved strawberry germplasms at our institute, 24 cultivars (lines) were identified as resistant to leaf blight (Fig. 1). Selection of resistant germplasms with superior horticultural traits from this pool is planned for use as breeding parents to improve disease resistance.

Soil disinfestation and rationalized fertilization

Healthy strawberry runner plants should be cultivated in healthy soil to produce high-quality strawberry. Crop rotation, soil disinfestation, and rational fertilization are essential components of strawberry IPM. Soilborne diseases such as Fusarium wilt could persist in the soil for several years, and if soil disinfestation is not implemented in affected fields, recurring outbreaks may occur and intensify over time. Additionally, rational fertilization not only supports normal plant growth but also prevents excessive nitrogen application, which might lead to increased pest and disease severity.

1. Soil disinfestation

The use of steam for controlling soilborne diseases can be traced back to the late 17th century. Soil steam disinfestation effectively suppresses soil pathogens and weeds while enhancing the vigor of crops grown on steamed soil (Fennimore & Goodhue 2016). Due to the low cost and high efficacy of fumigants such as methyl bromide (MB), steam disinfestation was mostly replaced in horticultural crops during the 1960s (Wilhelm 1966). This chemical method eliminates both beneficial and harmful microorganisms in the soil (Ristaino & Thomas 1997; López-Aranda *et al*. 2016; Holmes *et al*. 2020). However, it not only disrupts the physical and chemical structure of the soil but also poses risks to human health and the environment, leading to increasing restrictions and even bans on their use. Consequently, alternative methods that are relatively more environmentally friendly, such as steam applicators, have been developed (Fennimore & Goodhue 2016). In recent years, anaerobic soil disinfestation (ASD) has been introduced in California strawberry fields as an alternative method (Shennan *et al*. 2014). This approach involves adding organic matter to the soil and creating an anaerobic environment by sealing off oxygen, causing microbial proliferation and oxygen depletion, ultimately leading to the extinction of soilborne pathogens under anaerobic stress. Additionally, Japan has adopted the solarization-calcium cyanamide method for soil disinfestation (Kodama & Fukui 1979), primarily applied in greenhouses. During the high-temperature summer period, organic matter and calcium cyanamide are incorporated into the soil, which is then thoroughly moistened and covered with transparent plastic film. The disinfestation effect is achieved through heat accumulation from solar energy and the release of cyanamide during the decomposition of calcium cyanamide.

Currently, soil disinfestation methods for strawberry cultivation in Taiwan vary depending on the cultivation system. In elevated cultivation facilities, most growers cover the cultivation beds with plastic film during the summer to utilize solar heating for sterilization, while a few farms install heating systems for hot water disinfection. In open field soil-based systems, most farmers do not conduct soil disinfestation after the strawberry season but instead rotate to summer crops, while a small number practice fallowing with field flooding or apply calcium cyanamide during soil preparation (Chung & Wu 2017). In response to the widespread occurrence of Fusarium wilt in open field strawberry farms in recent years, our research team provided technical guidance to farmers who had not fallowed their fields for many years, implementing either anaerobic soil disinfestation or the solarization-calcium cyanamide method based on environmental conditions in 2023 and 2024. Significant improvements were observed in most field; however, some fields did not show notable effects. Apart from the potential

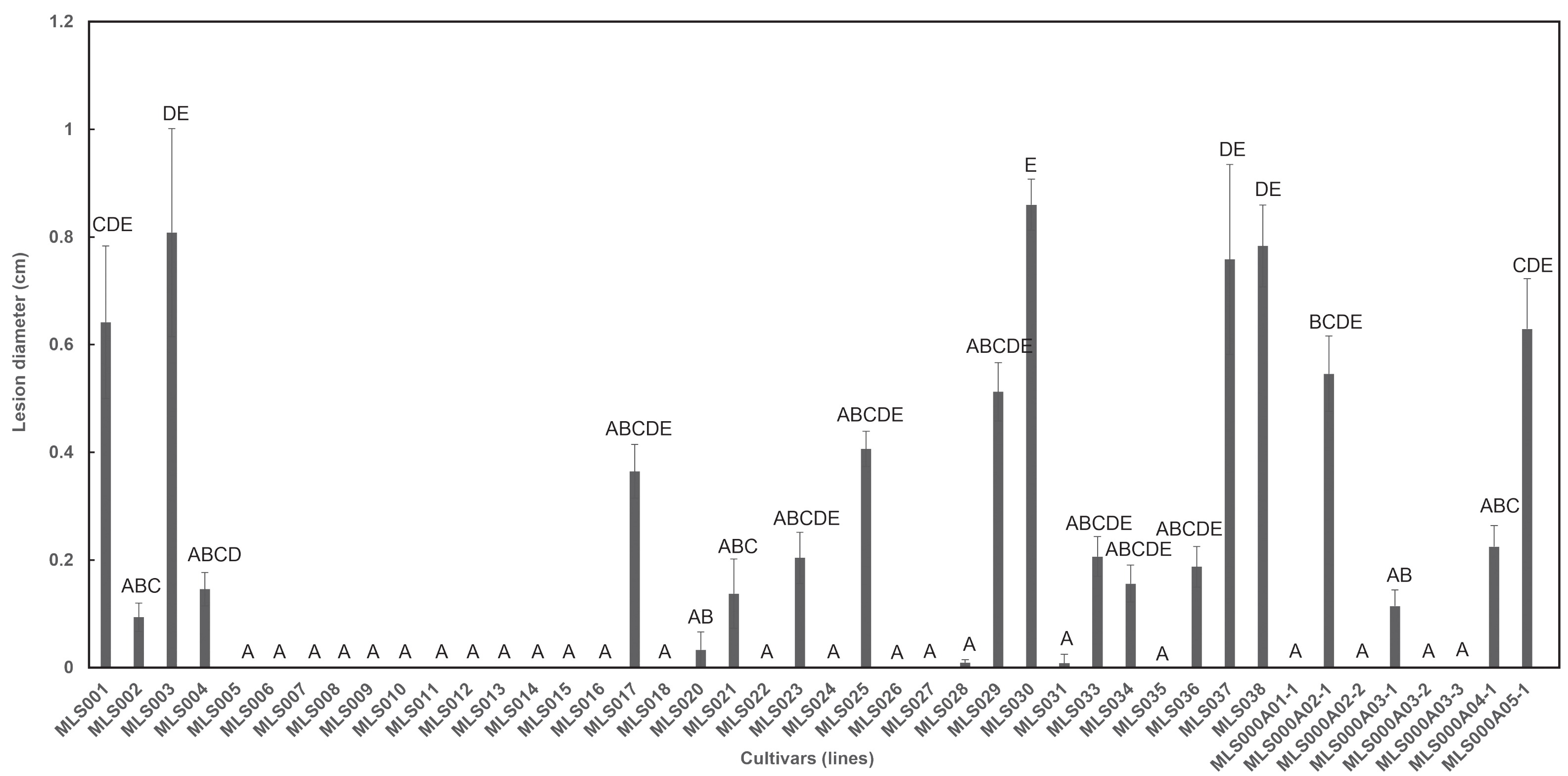

Fig. 1. Diameters of lesions on detached leaves of strawberry cultivars (lines) inoculated with *Neopestalotiopsis rosae* ML2147 at 7 d post inoculation. All bars are mean ± SEM. Different letters indicate statistically significant differences (Tukey-Kramer post hoc test, P value ≤ 0.05).

presence of infected planting materials, possible contributing factors include insufficient increases in soil temperature, inadequate soil moisture levels, and the potential for the pathogen to utilize organic matter added during anaerobic treatment (Yonemoto *et al*. 2006; Henry *et al*. 2020). These factors should be considered for future technical guidance and optimization of soil disinfestation strategies.

2. Rationalized fertilization

Strawberry farmers commonly apply large amounts of chicken waste compost, soybean fertilizer, or chemical fertilizers 4 wk before transplanting, with nitrogen application rates ranging from approximately 322 to 459 kg ha^{-1} (Table 1). To facilitate the evaluation of the impact of such fertilization rates, the nitrogen decision support system (Fink & Scharpf 1993) could be used to estimate the mineral nitrogen content of each independent component using a dynamic model (Table 2). In this system, N_0 represents the potentially mineralizable nitrogen, and k is the mineralization rate constant, which ranges from approximately 0.03 to 0.054 wk^{-1} at room temperature (Smith & Stanford 1971; Tsai 1993; Chuang *et al*. 1995). Under field conditions, the k value is not constant but rather a function of soil temperature and soil moisture (Mary & Recous 1994). The temperature relationship is expressed as $k_2 = k_1 \times Q_{10}$ $((T_2\text{-}T_1)/10)$, where Q_{10} is 4.0 (Sierra 2002). Regarding soil moisture, if rainfall before transplanting is insufficient, mineralization rate is limited by soil moisture deficit, and k is considered to be 0. After transplanting, irrigation stabilizes soil moisture, maintaining a constant k value. Based on the nitrogen application rate of 459 kg ha^{-1} commonly used by strawberry farmers (Table 1), if 50% of the nitrogen is mineralizable (Tsai *et al*. 1993), N_0 is estimated to be approximately 230 kg ha^{-1}, with an additional 60 kg N ha^{-1} from chemical fertilizers. Assuming the initial mineral nitrogen content in the soil before fertilization is 90 kg ha^{-1}, the changes in soil mineral nitrogen content following fertilization are illustrated in Fig. 2. Between the 4th and 8th wk after transplanting, soil mineral nitrogen (N residue) reaches its peak. According to the analysis of soil properties in severely affected strawberry fields in the Dahu region in 2023, when soil mineral nitrogen exceeds 135 kg ha^{-1}, soil electrical conductivity (EC) rises above the optimal threshold for strawberry (0.20 dS m^{-1}) (Fig. 3), making this period the most susceptible to growth disorders. After the 8th wk post transplanting, soil mineral

Table 1. Investigation results of soil properties and basal fertilizer application before strawberry transplanting in Dahu Township.

Soil properties	Mountain area (n = 34)[z]	Riverside (n = 69)[z]
pH	6.00 ± 0.89	6.22 ± 0.69
EC, dS m^{-1}	0.14 ± 0.07	0.14 ± 0.10
OM, g kg^{-1}	26.7 ± 8.51	21.5 ± 4.96
Av. N, mg kg^{-1}	15.7 ± 3.24	17.6 ± 5.42
CEC, cmol+ kg^{-1}	12.2 ± 3.54	8.8 ± 2.4
N application, kg ha^{-1}	459 ± 208	322 ± 132

[z] Data are mean ± standard deviation.

Table 2. Components related to soil mineral nitrogen content.

Components	Descriptions	Dynamic model
N plant	N uptake in plant	$N_{yield} \div (1 + A \times e^{-Bt})$
N loss	N loss by leaching, runoff or denitrification	$N_s \times C \times (1 - e^{-kt})$
N humus	N from mineralization of humus and crop residues	$N_H \times (1 - e^{-kt})$
N manure	N from mineralization of oganic fertilizer	$N_0 \times (1 - e^{-kt})$
N chemical	N from chemical fertilizer	
N soil	Mineral N at begin of cultivation	
N residue	N soil + N chemical + N manure + N humus- N loss- N plant	

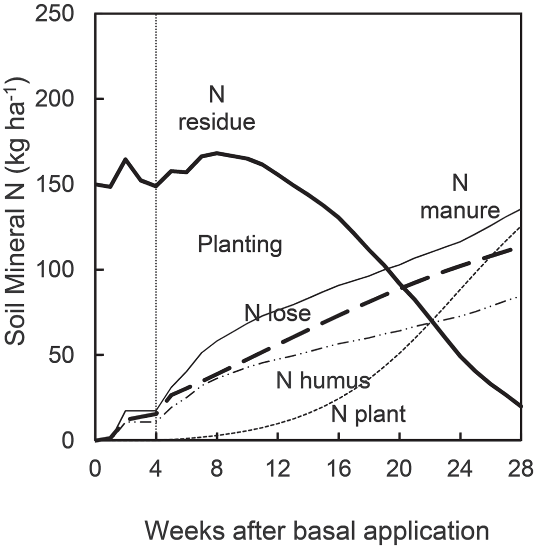

Fig. 2. Diagram of changes in soil mineral nitrogen content after base fertilizer application.

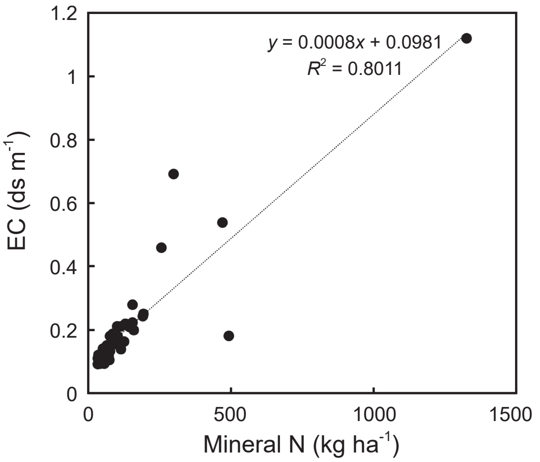

Fig. 3. Relationship between soil mineral nitrogen and soil electrical conductivity (EC) in severely diseased strawberry fields in Dahu Township, 2023.

nitrogen gradually decreases, aligning with the timing of topdressing applications by farmers.

Using meteorological data on soil temperature (at a 10 cm depth) and rainfall collected during the strawberry cultivation period in the Dahu region from 2022 to 2024, the changes in soil mineral nitrogen content under different transplanting dates and fertilization rates were further evaluated, as shown in Fig. 4. Higher N0 values resulted in greater soil mineral nitrogen levels exceeding 135 kg ha^{-1} during the early transplanting stage, with annual variations, showing the lowest levels in 2024. Fields transplanted in late September exhibited a higher degree of soil mineral nitrogen excess compared to those transplanted in late October. To mitigate early transplanting risks in strawberry production, adopting a varying fertilizer practice is recommended (FASS 1995). As shown in Fig. 4, lower N0 values combined with liquid fertilizer fertirrigation resulted in a lower risk of excessive soil mineral nitrogen accumulation. Therefore, soil analysis before basal fertilization is recommended to determine a reasonable nitrogen application rate, followed by fertigation with liquid fertilizers from transplant establishment until the end of production to maintain soil mineral nitrogen within an optimal range.

Cultivation management techniques in response to climate change

In recent years, strawberry cultivation has faced various threats and challenges due to climate change. High temperatures during the propagation stage of the runner plants have caused heat stress and inhibited growth. Additionally, intermittent heavy rains have facilitated the spread of diseases. During the fruiting period, early-stage high temperatures combined with excessive fertilization result in poor root development, increasing the incidence of soilborne diseases and disrupting the continuity of strawberry flowering. In addition to preventive measures such as facility-based runner plant propagation, delaying transplanting in soil-based areas, and rationalized fertilization, the following section will describe the cultivation management techniques currently being developed by our research team. Through the implementation of IPM strategies, we aim to address the challenges posed by climate change, reduce the spread of diseases during the runner plants stage to ensure the propagation of high-quality nurseries, and maintain consistent flowering for stable production of high-quality strawberry fruit during the fruiting period.

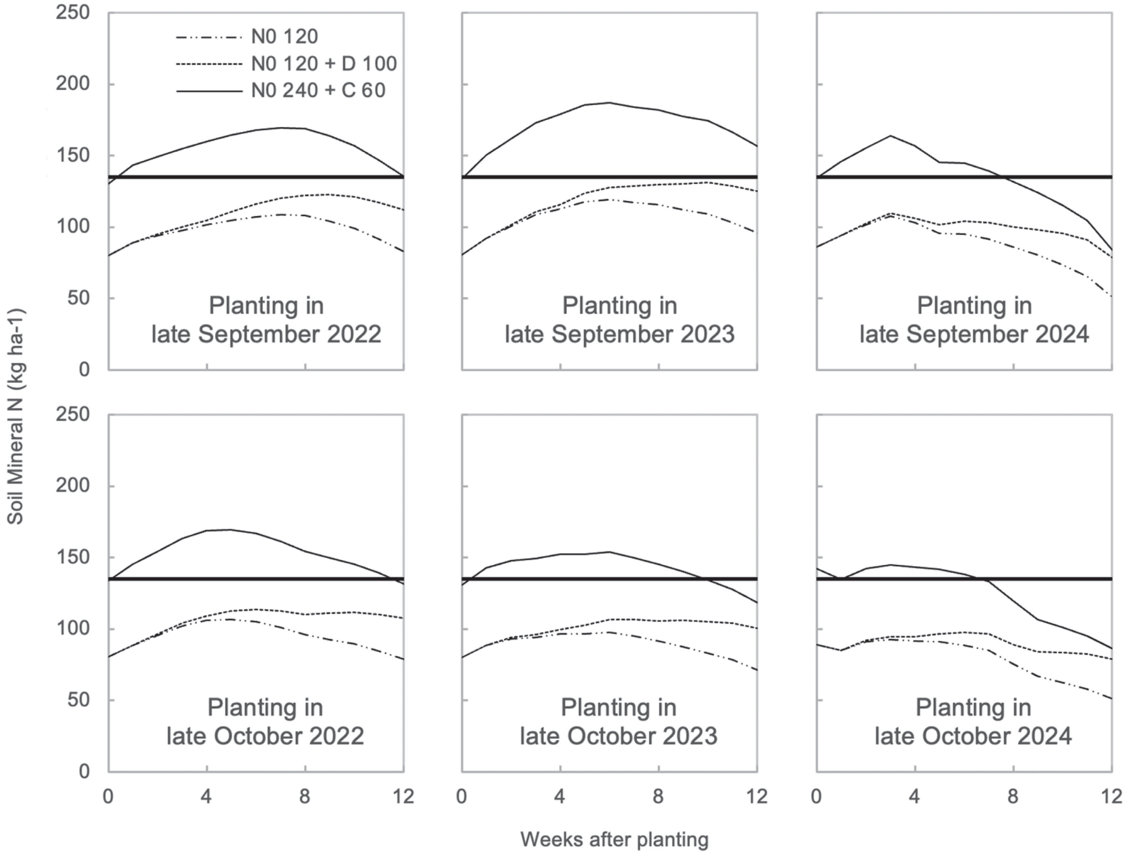

Fig. 4. The changes in soil mineral nitrogen content after transplanting during different strawberry planting periods and fertilization rates from 2022 to 2024 in Dahu Township. Data were estimated based on 10 cm-depth soil temperature and rainfall data. The horizontal line represents the soil mineral nitrogen threshold (135 kg ha^{-1}). N0 120: Organic fertilizer with 120 kg N ha^{-1}; N0 120 + D100: Organic fertilizer with 120 kg N ha^{-1} combined with liquid fertilizer applied at 100 kg N ha^{-1}; N0 240 + C60: Organic fertilizer with 240 kg N ha^{-1} combined with chemical fertilizer at 60 kg N ha^{-1}.

1. Strategies against adverse conditions during the runner plant propagation period

Strawberry runner plants are propagated using paper pots in Shizuoka Prefecture, Japan. The temperature inside the paper pots is lower, and inflorescence initiation occurs about 7 d faster. The blooming time of the terminal flowers and the harvest time of the fruit are approximately 2 wk earlier than when propagated in plastic pots. Additionally, using paper pots and plastic pots with the tray water supply method for runner plant propagation effectively prevents the spread of anthracnose rot, compared to conventional overhead irrigation (Ikari 2010). Lower root temperature (12℃) promotes vegetative growth in strawberry, increasing petiole length, leaf area, and fresh leaf weight. In contrast, higher root temperature (24℃) suppresses vegetative growth (Lieten 1997).

During the runner plant propagation period, our research team evaluated four types of nursery containers: black plastic pot, transparent plastic pot, pulp cup, and plastic plug. Soil thermometers were used to record the temperature inside the containers at 9:00, 12:00, and 15:00 from July to August. The results showed that at all three time points, the

temperature inside the pulp cup was the lowest among the four containers, while the plastic plug had the highest temperature. There was no significant difference in temperature between the two plastic pots at any time point, indicating that the color of plastic pots might have minimal influence on the medium temperature. Regarding the growth of the runner plants, black plastic pot and pulp cup performed better in above-ground growth, with the black plastic pot having significantly higher fresh and dry shoot weights than the other three containers. Root morphology varied among containers: black plastic pot exhibited obvious root circling, transparent plastic pot showed minimal root circling with some scattered thick roots, pulp cup had no root circling, and plastic plug allowed natural air pruning at the bottom, with roots adhering to the tube walls. However, root biomass showed no significant difference among different treatments. After transplanting, there was no significant difference in the time to first flowering among runner plants from four containers, but black plastic pot resulted in the highest yield. Among the four nursery containers, pulp cup maintained the lowest temperature under high summer temperatures, while black plastic pot showed the best runner plant growth and post-transplant yield (unpublished data).

2. Strategies against adverse conditions during the fruit production stage

Most major strawberry-producing countries select appropriate cultivars based on climate conditions and cultivation methods while adjusting the production period in response to climate change. In some temperate European countries and Japan, greenhouse cultivation has been developed to mitigate temperature stress. For example, only a minimal amount of open-field strawberry cultivation remained in Japan after 1995, and the area dedicated to soilless elevated substrate cultivation has continued to expand since 2000 (Yoshida 2013).

For greenhouse cultivation under high-temperature stress, in addition to comprehensive environmental temperature control, localized cooling techniques have been developed. For example, partial temperature control, such as placing plastic tubes with running cooling water next to the crown tissue, could effectively lower temperature and promote flowering (Hidaka *et al*. 2017). Additionally, ensure ventilation underneath the culture system to cool down the culture medium by the latent heat of water evaporation (Ikeda *et al*. 2007). For large scale open-field cultivation, using different mulching materials to reduce heat stress. In California, an aluminum-coated treatment was applied to the center of black plastic mulch to increase reflectivity and reduce high temperatures during the early transplanting period, while the sides remained black to retain heat during winter. Compared to the control, this treatment reduced root zone temperature by up to 3.1℃ and increased yield (Deschamps & Agehara 2019).

The primary method for mitigating high temperature stress of strawberry cultivation remains soilless elevated substrate cultivation in Taiwan. For soil-based cultivation, our research team is currently conducting trials to evaluate alternative mulching materials as substitutes for the conventional silver-black plastic mulch to alleviate excessive heat accumulation on the surface of the raised bed. Preliminary experiments have shown that certain alternative materials can reduce surface temperature by up to 4℃ compared to the control during peak noon temperatures (unpublished data). Further multi-year trials and cost benefit analyses are required to assess the feasibility of widespread adoption.

Strategies for controlling small pests

Strawberry suffers from two types of thrips. One is the leaf thrips, primarily the yellow tea thrips (*Scirtothrips dorsalis* Hood) or the southern yellow thrips (*Thrips palmi* Karny), which usually hide at the base of the petiole. They feed on the leaves through rasping, causing the leaf veins to turn dark brown. Preventive

or control measures should be implemented before transplanting to avoid the introduction of pests into the planting field along with the runner plants. The other type is the flower thrips (*Frankliniella intonsa* Trybom), which rasps the flowers with its feeding mouthparts. If the flowers are affected, they cause fruit deformities, hardening, and rust-colored discoloration, rendering the fruits unmarketable. Two-spotted spider mite (*Tetranychus urticae* Koch), commonly known as the red spider mite, is another small pest of strawberry. The damaged leaves develop tiny yellow spots, which then turn reddish-brown and spread across the entire leaf, leading to leaf wilting and drop.

Thrips and spider mites are adept at hiding and could rapidly proliferate during high-temperature periods, causing farmers to miss the optimal timing for pest control. Additionally, control agents often fail to make direct contact with the pests, leading to poor efficacy, while frequent applications of chemical pesticides contribute to resistance development (Price *et al*. 2002; Van Leeuwen *et al*. 2010; Bernardi *et al*. 2013; Lahiri *et al*. 2022). Therefore, it is necessary to implement IPM strategies, such as using colored sticky cards for monitoring or reducing pest density, applying environmentally friendly materials, and natural enemies for pest control. A Dutch biocontrol company (Koppert) has developed specialized packaging, including sachets and hang sachets containing predatory mites *Amblyseius swirskii* and *Neoseiulus cucumeris*, to target thrips larvae (Arthurs *et al*. 2009; https://www.koppert.com). The company has also developed predatory bugs of *Orius* spp. to control adult thrips (Zuma *et al*. 2023; https://www.biobest.com) and beneficial nematodes *Steinernema feltiae* to combat thrips pupae that burrow into the soil (Renkema *et al*. 2018). A Belgian biocontrol company (Biobest Shouguang Biological Control Ltd.) has also employed predatory mites for strawberry spider mite management. They recommend using sachets containing *N. californicus* at the early stages of strawberry cultivation as a preventive measure, applying 1–2 sachets per square meter at entry points where spider mites are likely to invade. *N. californicus* is a generalist predatory mite that, when used with sachets containing supplemental food, could survive even in low spider mite populations, providing long-term pest control (Fraulo & Liburd 2007). *Phytoseiulus persimilis*, on the other hand, is a more specialized predatory mite that is used as a curative treatment when spider mite populations begin to increase significantly in the field (Zhao *et al*. 2023; https://www.biobest.com).

In Taiwan, *N. longispinosus* is recommended for controlling strawberry two-spotted spider mites. During the early stages of transplanting, it is evenly released at a density of 5,000–10,000 individuals per 0.1 ha every 2 wk, and after three consecutive applications, the incidence of spider mites can be reduced while also decreasing the use of chemical pesticides (Shih 2010; unpublished data). The non-exclusive licensing of the production and release technology for this predatory mite has already been announced. Once commercialized, its release following the IPM strategy will help address the frequent pesticide application in strawberry fields, which often fails to achieve effective spider mite control. For managing flower thrips in strawberry, *Orius strigicollis* is used as a biological control agent. When thrips infestation is observed during the flowering period, natural enemies are released at a density of 5,000–20,000 individuals per 0.1 ha, effectively reducing thrips damage to strawberry fruits (Lin *et al*. 2017). Additionally, if strawberry is affected by broad mites, *N. barkeri* has been developed domestically as a biocontrol agent. In severely infested plants, a density of 50–100 individuals per m^2 is applied weekly, which has been shown to restore normal plant growth (https://goodfarms.neophoen.com).

In addition, to reduce the usage of chemical pesticides, Agricultural Chemicals Research Institute (ACRI, MOA) has developed a rubber-cap-type thrips alarm pheromone. Farmers could begin hanging these pheromone dispensers at the early stages of strawberry cultivation before

thrips populations are established. The rubber cap should be placed upside down approximately 1 meter above the strawberry plants, allowing the pheromone to diffuse downward and outward through air circulation, causing thrips to become restless, thereby reducing their residency and oviposition rates, ultimately achieving a control effect. The recommended hanging density is one pheromone dispenser every 2 m, with approximately 300 units per 0.1 ha, and each dispenser remains effective in the field for about 6 mo. During the implementation of IPM programs for strawberry, the thrips alarm pheromone has been successfully promoted. In addition to its small size, ease of installation, and long-lasting effect, the key to its success lies in effectively reducing thrips population density, improving fruit quality, and lowering costs associated with purchasing and using pesticides. However, this pheromone is still in the trial promotion stage in Taiwan and has not yet been registered as a pesticide, requiring further efforts from governmental agencies for official approval.

Although control agents such as natural enemies and pheromones are more costly compared to chemical pesticides, when integrated with IPM strategies like population density monitoring and early prevention, they ultimately offer greater advantages in terms of production costs, economic benefits, and environmental sustainability over the frequent application of chemical pesticides.

AI and decision support tools in IPM

1. Image recognition and early warning system for diseases and pests in strawberry

In recent years, due to global climate change, modern technology is needed to assist farmers to manage increasingly severe outbreaks of diseases and pests. The University of Florida has developed a strawberry disease early warning system (Strawberry Advisory System; SAS) (Montone *et al*. 2016; Kondaparthi *et al*. 2024). The system automatically collects temperature through weather monitoring stations and combined with leaf wetness duration (LWD) data, which is estimated using mathematical models. These combined data are then used in formulas to calculate the infection index (INF). Based on the INF values, the risk is classified into three levels- low, medium, and high- and color-coded accordingly. This system provides recommendations on whether farmers should apply fungicides. Research results indicate that by using the information of the early warning system and applying control materials when necessary, fungicide application can be reduced by about 50%, with no negative impact on disease control and yield. To facilitate use by farmers, SAS also developed a mobile app version, simplifying the interface and offering disease risk levels and forecasts. It provides fungicide application suggestions through a simple questionnaire to reduce the risk of resistance development. In Lithuania, a European country, field weather monitoring data have been used to develop a system to predict the occurrence period of gray mold. The system, iMETOS® (Rasiukevičiūtė *et al*. 2019), is based on disease early warning and fungicide application patterns. In experimental fields using the system, the yield was higher than in the control area (which received no treatment), and the proportion of fruit rot was lower. Compared to conventional fungicide application areas, no differences were observed in yield or fruit rot proportions. South Korea, one of Asia's major strawberry-producing countries, has established a data integration and analysis platform (Farm as a Service, FaaS) (Kim *et al*. 2018) using field environmental big data. This platform predicts the occurrence of strawberry gray mold based on field temperature and humidity conditions, offering better pest management strategies within smart farming, in addition to cultivation management practices such as fertilization and irrigation.

Our research team, in collaboration with the Department of Plant Pathology and Microbiology, the Department of Agronomy, and the Department of Biomechatronics Engineering at National Taiwan University, has developed

a "Strawberry Pest and Disease Recognition Robot and Disease Early Warning System". For disease early warning, five experimental fields were established in major strawberry production areas, equipped with a weather station to collect climate data. Disease severity was assessed through regular field surveys. Using logistic regression combined with field survey data on strawberry disease severity and microclimate data, two disease prediction models have been established. By analyzing the past 7 d daily high temperatures and low humidity data, these models can predict the likelihood of anthracnose rot and leaf blight worsening within the next 7 d, allowing farmers to monitor disease development and implement timely control measures. For anthracnose rot, the model exhibited an AUC of 0.918 and an accuracy of 0.811; for leaf blight, the model's AUC was 0.951 with an accuracy of 0.839 (unpublished data). However, compared to the cultivation models with other countries, strawberry farms typically have smaller individual cultivation areas in Taiwan, and the accuracy of disease warning systems is significantly influenced by factors such as topography, microclimate, and cultivation management practices. For the successful implementation of precise applications in the future, it is necessary to accumulate years of survey data and validation. For image recognition, SqueezeNet was adopted as the model architecture, applying deep learning and utilizing over ten thousand field images of pests and diseases to train and test the recognition model. After training, the model can identify five types of strawberry pests and diseases-anthracnose rot, leaf blight, angular leaf spot, damage of spider mite and lepidopteran larval-with an overall accuracy rate of 98.3% (Chiu *et al*. 2022). This system will help farmers distinguish between visually similar symptoms on strawberry leaves. By integrating the image recognition system, disease early warning system, and control recommendations into a user-friendly LINE bot interface, farmers will be able to access relevant information through commonly used messaging apps (Fig. 5). This approach enables fast and accurate pest and disease identification, promotes better pesticide use practices, reduces chemical pesticide application, and enhances strawberry quality in the future.

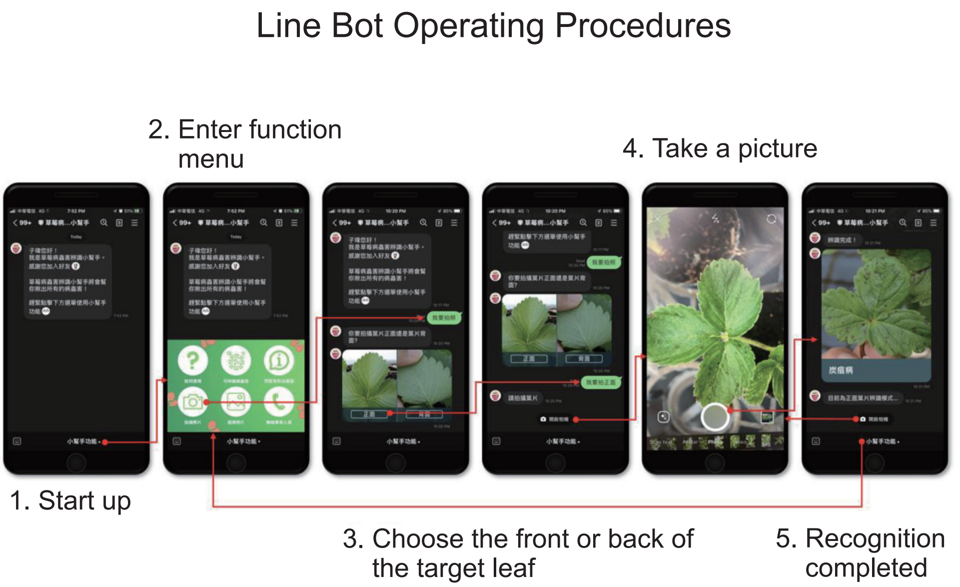

Fig. 5. The Line bot could assist farmers in quickly identifying major strawberry pests and diseases in the future.

Case studies and field implementation in Taiwan

1. Case study on the implementation of IPM technology in severely Fusarium wilt infested soil-based fields in 2023 to 2024

Case I: This case involves a strawberry farmer in Dahu Township, Miaoli County, whose field suffered severe plant loss and wilting at the end of 2022, with a loss rate of 100%. On-site investigation confirmed that the issue was caused by Fusarium wilt infected runner plants. Therefore, starting in June 2023, our research team provided assistance in monitoring pests and diseases during the runner plants period, guiding the farmer in identifying pest and disease types, implementing thorough field sanitation, and determining critical periods for control. During cultivation period in 2023, soil analysis was conducted before and after land preparation to assess soil fertility. Based on the analysis results, appropriate amounts of organic compost were recommended as base fertilizer to prevent excessive EC. After transplanting, different liquid fertilizers were supplied according to the various growth stages of strawberry along with the application of beneficial microorganisms, including *Bacillus* spp. and *Trichoderma* spp.. As a result, the replanting rate was successfully reduced to below 10%, from an economic perspective, a 0.4 ha plot incurred a loss of approximately 700,000 to 800,000 NTD in 2022, while in 2023, the net income from the same plot reached around 1,700,000 to 1,800,000 NTD, demonstrating significant effectiveness.

Case II: The assisted farm is located approximately 300 m from the tourist attraction "Dahu Winery" in Dahu Township, Miaoli County. The farmer suffered from a significant yield reduction over the past two years. After transplanting in late September to early October 2023, disease symptoms began appearing in mid-November, prompting the farmer to seek assistance from our research team. On-site investigation confirmed that Fusarium wilt was the primary cause of damage. Soil sampling and analysis indicated multiple signs of excessive fertilization. Consequently, for that growing season, the farmer was advised to apply biopesticides targeting Fusarium wilt and to cease additional fertilization to mitigate field damage. During the summer of 2024, the farmer implemented partial fallow management and, in early July, conducted soil disinfestation using the solarization-calcium cyanamide method under our guidance. Temperature loggers were buried at a soil depth of approximately 15 cm to monitor the effectiveness of disinfestation. After 52 d, the cumulative data confirmed that the soil temperature exceeded 35℃ for 652 h, with 186 h exceeding 40℃. Following soil disinfestation, base fertilizer and beneficial microorganisms including *Bacillus* spp. and *Trichoderma* spp. were applied according to our recommendations, and runner plants propagated by farmers regularly guided by our research team were transplanted in late October. A neighboring field that likewise suffered from severe Fusarium wilt in the prior period was utilized as the untreated control for comparison. The field showed an approximate 8% replanting rate (due to anthracnose rot), which was a significant improvement compared to the nearly 65% Fusarium wilt incidence observed in the adjacent untreated field. As of the end of February 2025, fruit production was at its peak. Although disinfestation increased costs, replanting costs were reduced by 86%. The demonstration area reduced fruit yield loss by 74% and achieved a 382% increase in sales revenue when compared to the control area. These results demonstrate the economic benefits of integrating IPM strategies.

Limitations, knowledge gaps, and future directions

Strawberry is a temperate crop, and its cultivation in Taiwan with a subtropical climate faces multiple pest and disease threats, further compounded by the challenges of climate change. Collaboration between research institutions and farmers is essential to effectively address industry issues with developed technologies.

Strawberry healthy runner plants propagation

system has been implemented since 1990 in Taiwan (Lee 1993), but it garnered more attention in the past decade due to the severe occurrence of diseases. The diseases not only led to a significant increase in replanting rates during the early stages of transplanting, raising input costs, but also directly affected the yield per unit area. To reduce the risk of disease spread due to latent infections from self-retained mother plants, our research team has guided farmers in using tissue culture plants that have been acclimatized in a controlled facility environment (G1). In recent years, several companies have invested in the production of G1 to provide farmers with a source of mother plants for runner plants propagation. However, quality control during the acclimatization stage in greenhouses and the management practices after transplanting to the field still require assistance through education, training, and on-site guidance to help growers and farmers improve the quality of runner plants and gradually implement professional runner plants propagation.

In recent years, climate change has posed significant challenges to strawberry production, particularly during summer, with high temperature, heavy rainfall, and multiple rainfall events within a single day, as well as high temperature during the early transplanting stage. These are unavoidable issues for the strawberry industry. To mitigate the risk of pest and disease outbreaks triggered by environmental stress and to stabilize fresh fruit yield, it is essential to adopt adaptive cultivation management techniques, invest in protective facilities, and implement localized cooling methods for plants (Morton *et al*. 2017; Menzel 2023, 2024).

Strawberry production in Taiwan has long been dominated by large-scale monoculture of a single cultivar. However, as a high-value crop with significant agritourism benefits, strawberry cultivation can attract consumer traffic during the production season, stimulating the surrounding tourism industry. Therefore, diversifying cultivars is recommended to extend the strawberry season by utilizing differences in flowering and fruiting times among cultivars while also reducing the risk of pest and disease outbreaks associated with monoculture. For instance, ‘Taoyuan No. 1’, the dominant cultivar since its promotion in 1990, has been cultivated for nearly 35 years and is highly susceptible to anthracnose rot and powdery mildew. Since 2019, the rapid expansion of ‘Xiang-Shui’, which now accounts for approximately 80% of the cultivation area, has resulted in the emergence of leaf blight as the primary disease. Compared to ‘Taoyuan No. 1’, ‘Xiang-Shui’ exhibits higher resistance to anthracnose rot and powdery mildew. The most environmentally friendly and labor-saving approach to managing disease issues is the cultivation of disease-resistant cultivars. However, strawberry breeding in Taiwan still relies primarily on traditional hybridization, with breeding goals focused on economic yield and desirable horticultural traits as the main selection criteria. Disease resistance is primarily assessed through field observations, but the lack of effective early screening, coupled with the influence of climate and cultivation conditions on disease resistance performance, may lead to reduced resistance in newly released cultivars. This could impact the willingness of farmers to propagate and cultivate new cultivars.

In recent years, in addition to continuous efforts by research institutions, farmers and nurseries have also been actively selecting and breeding new strawberry cultivars. If more breeding-related resources can be allocated, future strawberry cultivars intended for commercial cultivation could be evaluated for resistance or susceptibility to major diseases through the screening platform gradually established by our research team. This would ensure that, in addition to cultivation characteristics, fundamental data on disease resistance or susceptibility are available during the promotion of new cultivars, facilitating the development of comprehensive IPM strategies and proactive disease management. In the future, integrating marker-assisted selection with high-throughput plant phenotyping technologies is expected to reduce the need for extensive field selection while accelerating the breeding process,

aligning with the long-term goal of developing disease-resistant strawberry cultivars.

Accurate identification of pests and diseases is a prerequisite for effective strawberry IPM implementation, as proper diagnosis ensures targeted and precise pesticide application. Our research team will continue collaborating with interdisciplinary research groups to develop computer programs trained through machine learning and deep learning for recognizing strawberry pests and diseases. Additionally, by integrating expert decision support systems, we aim to provide accurate and appropriate control strategies based on different pest and disease occurrences under varying environmental conditions, crop stages, and field information. This approach will assist farmers in accurately identifying pests and diseases in real time without expert assistance, enabling successful pest management while reducing the likelihood of incorrect pesticide applications.

Differences in field environmental conditions, cultivation management practices, and strawberry cultivars lead to variations in pest and disease occurrences, resulting in different levels of economic loss. Farmers require corresponding preventive or control measures, while technologies developed by research institutions must be efficiently and rapidly disseminated. Close collaboration with locally trained plant doctors is a key factor in achieving this goal. For example, in 2024, our research team worked with regional plant doctors to promote soil disinfestation practices in soil-grown strawberry fields. Our team developed the extension strategy and implementation timeline, conducted regular meetings to discuss and monitor progress, and established communication groups to provide timely field-specific recommendations. Additionally, we led plant doctors in hands-on soil disinfestation procedures, field diagnostics, and post-transplanting assessments of plant growth to determine appropriate liquid fertilizer application frequencies and irrigation volumes. The outcomes of these efforts were showcased through four demonstration and observation events held from late 2024 to February 2025, with over 300 participants. These events not only facilitated in-depth discussions between farmers and the implementation team but also encouraged neighboring farmers to adopt similar practices.

CONCLUSION

The current strawberry yield in Taiwan is approximately 17.96 metric tons per hectare, which is about 56% of the per-unit-area yield in Japan, a well-known strawberry-producing country favored by consumers. Besides inherent climatic limitations, this yield gap can potentially be narrowed through the introduction of new cultivars as breeding materials, improvements in cultivation management techniques, and the implementation of the IPM strategies for strawberry pests and diseases developed and promoted by our research team (Fig. 6). These measures aim to mitigate the risks posed by natural disasters and pest infestations, enhance strawberry yield and quality, increase income of farmers, and meet consumer market demand while simultaneously achieving ecological benefits through environmentally friendly practices.

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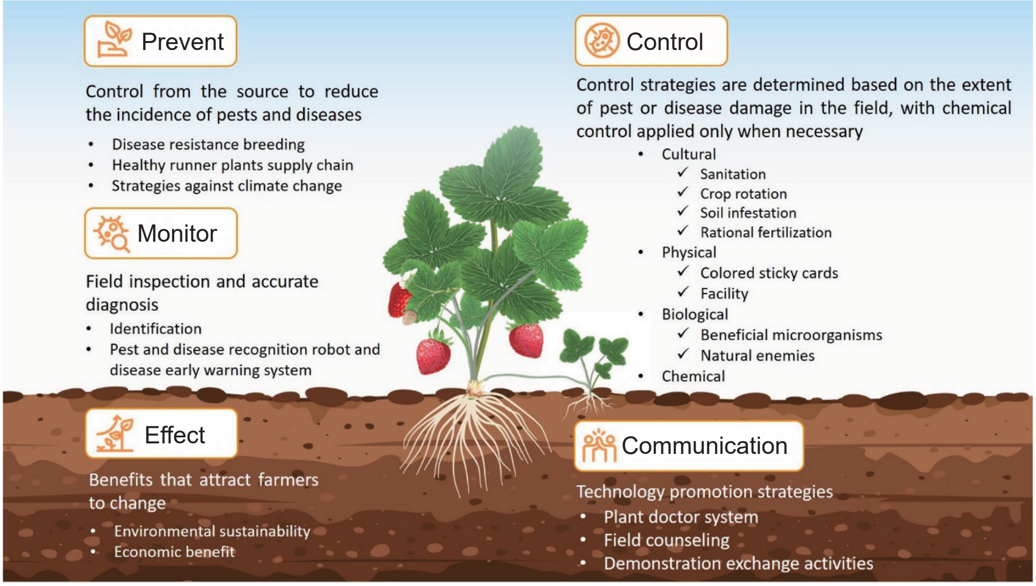

Fig. 6. Integrated pest management (IPM) strategies for strawberry cultivation.

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臺灣草莓生產之整合性病蟲害管理策略：技術創新與實施挑戰之綜述

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摘要

鐘珮哲、賴巧娟、葉人豪、蔡正賢、李怡蓓、鄭哲皓、呂秀英。2026。臺灣草莓生產之整合性病蟲害管理策略：技術創新與實施挑戰之綜述。台灣農業研究 75(1):1–26。

草莓為極具經濟價值之作物，國內栽培面積約 586 ha，主要產區位於苗栗縣 (約佔總生產面積 89.1%)，每當草莓生產期間總是吸引大批觀光客前往，帶動周邊產業發展。近十多年來草莓產業面臨病蟲害威脅、品種更迭及氣候變遷等挑戰，栽培難度逐年增加。本研究團隊以整合性病蟲害管理 (integrated pest management; IPM) 預防勝於治療的基礎，依草莓不同栽培階段與短中長期目標執行以下研發輔導策略：(1) 建立國內草莓健康種苗供應鏈，開發重要特定病害檢測技術落實於草莓種苗病害驗證作業須知，並輔導產業朝向專業育苗分工發展；(2) 建立重要病害耐 (感) 受性篩選平臺，確認國內種原對病害之耐 (感) 受性，並將結合分子標誌輔助育種與高通量植物表型分析技術，未來可望減少大量田間選拔工作並且提升育種速度；(3) 因應氣候變遷，調適育苗期與產果期栽培管理策略以降低高溫對草莓生產之影響；(4) 草莓定植前輔導農民進行土壤消毒、依土壤分析數據施用基肥及定植後合理化追施液肥；(5) 與跨領域團隊合作利用機器學習或深度學習等訓練電腦程式辨識草莓病蟲害種類，同時結合專家決策輔助系統幫助農民即時且正確地辨識病蟲害並成功防治。期望藉由 IPM 技術的推廣，逐步提升國內草莓產量與品質，不僅增加農民收益且友善環境同時滿足消費市場需求。

關鍵詞：健康種苗、抗病育種、氣候變遷、土壤消毒、深度學習。

投稿日期：2025 年 3 月 11 日；接受日期：2025 年 10 月 29 日。
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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):27–49 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0002

Feature Article

Research and Development of Pathogenic Microorganisms for the Control of Major Agricultural Pests

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Abstract

Chang, S. C., C. C. Lee, and M. Y. Chiang. 2026. Research and development of pathogenic microorganisms for the control of major agricultural pests. J. Taiwan Agric. Res. 75(1):27–49.

Pathogenic microorganisms play a crucial role in agricultural pest management by suppressing pest populations through parasitism, infection, or toxin production. These microorganisms offer a sustainable and environmentally friendly alternative to chemical pesticides, contributing to integrated pest management (IPM) strategies. Insect-pathogenic microorganisms have been extensively studied and widely applied in biological control. These include bacterial pathogens such as *Bacillus thuringiensis*, entomopathogenic fungi (EPFs) like *Beauveria bassiana*, *Metarhizium anisopliae*, and *Cordyceps javanica*, as well as insect-pathogenic viruses such as nucleopolyhedrovirus (NPV). Due to their high specificity towards insect pests, environmental compatibility, and potential to reduce reliance on chemical pesticides, these microbial agents have become an integral part of IPM strategies. In recent years, advancements in genomics and biotechnology have significantly accelerated the selection of highly effective pathogenic strains and the elucidation of their virulence mechanisms. Whole-genome sequencing and transcriptomic analyses have facilitated the precise identification of key genes involved in pathogenicity, enabling further exploration of toxin production, parasitic capabilities, and host adaptability. Additionally, studies on specific enzymes, such as chitinases and proteases, as well as secondary metabolites like fungal toxins and bacterial exoproteins, have contributed to enhancing the infection efficiency and insecticidal activity of these microorganisms, expanding their application potential. Despite the numerous advantages of microbial control, including environmental safety, high specificity, and minimal residue concerns, several challenges remain in practical applications. These include the environmental adaptability of microbial agents, the evolution of host resistance, and issues related to formulation stability and field efficacy. Overall, microbial control technologies play a vital role in modern agriculture. With continuous advancements in biotechnology and the development of novel application strategies, these technologies are expected to become a cornerstone of pest management, promoting sustainable agriculture and global food security.

Key words: Entomopathogens, Microbial pesticides, Bacteria, Fungi, Viruses.

INTRODUCTION

Pest infestations are an inevitable challenge in agricultural cultivation. Synthetic chemical pesticides have long been the primary tool for pest management due to their rapid action, high efficacy, and ease of application. They also remain an essential component of integrated pest management (IPM). However, concerns over their environmental toxicity, negative impacts on non-

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target organisms, and the increasing prevalence of pesticide resistance (Ku *et al*. 1994; Tabashnik 1994; Gao *et al*. 2012; Adesanya *et al*. 2018; Lin 2019; Jensen 2000; Bird *et al*. 2022; Bass & Nauen 2023) have led to a growing emphasis on the use of microbial pathogens for pest control.

In natural ecosystems, insects encounter a diverse range of microorganisms, including environmental microbes and endogenous symbionts. Some of these microbes can infect and cause disease in insects, ultimately leading to their death. Others, particularly endophytic microorganisms, play dual roles by suppressing plant pathogens and promoting plant growth. The most commonly used microbial pathogens for pest management fall into three main categories: bacteria, fungi, and viruses.

This review provides an overview of the applications of key microbial pathogens in agricultural pest management, highlighting their advantages as well as the challenges associated with their implementation. The scientific names of bacteria, fungi, and insects mentioned in this review follow the current valid nomenclature according to the International Code of Nomenclature of Prokaryotes (Parker *et al*. 2019), the International Code of Nomenclature for algae, fungi, and plants (Turland *et al*. 2018), and the International Code of Zoological Nomenclature (ICZN 1999), respectively. Taxonomic information was verified using the List of Prokaryotic names with Standing in Nomenclature (LPSN, https://lpsn.dsmz.de), Fungal Names (https://nmdc.cn/fungalnames), Catalogue of Life (https://www.catalogueoflife.org), and the Global Biodiversity Information Facility (GBIF, https://www.gbif.org).

BACTERIAL MICROBIAL AGENTS IN AGRICULTURAL PEST BIOCONTROL

Bacteria-based microbial formulations are widely used in agricultural pest control. Their production and field performance- affected by drying, heating, storage, humidity, temperature, and ultraviolet (UV) exposure- largely depend on the bacteria's ability to form spores. Pathogenic bacteria are therefore classified into three groups: (1) endospore-formers, such as *Bacillus*, *Clostridium*, and *Paenibacillus*; (2) exospore-formers, e.g., *Streptomyces*; and (3) non-spore-formers, including *Pseudomonas*, *Photorhabdus*, *Chromobacterium*, *Xenorhabdus*, *Serratia*, and *Yersinia* (Beskrovnaya *et al*. 2021; Sabbahi *et al*. 2022).

Bacillus thuringiensis

Bacillus thuringiensis Berliner, 1915 (Bt) belongs taxonomically to the phylum *Bacillota*, class *Bacilli*, order *Caryophanales*, family Bacillaceae, and genus *Bacillus*. Bt is a Gram-positive soil bacterium capable of producing insecticidal δ-endotoxins, which exhibit high lethality against various agricultural pests, including insects from the orders Lepidoptera, Coleoptera, and Diptera.

Under favorable environmental conditions, Bt spores germinate, and vegetative cells proliferate. However, under unfavorable conditions, during the stationary phase, Bt forms parasporal crystalline inclusions composed of Cry and Cyt proteins (Suzuki *et al*. 2004). Additionally, during the vegetative growth phase, Bt synthesizes and secretes insecticidal toxins into the growth medium, such as vegetative insecticidal proteins (Vip) and secreted insecticidal proteins (Sip) (Palma *et al*. 2014).

Cry proteins (δ-Endotoxins)

Cry proteins, also known as δ-endotoxins, are pore-forming toxins (PFTs) that are water-soluble proteins. These proteins undergo structural changes that allow them to insert into the host membrane, forming lytic pores that disrupt the integrity of the host cell membrane (Gonzalez *et al*. 2008; Sanchis & Bourguet 2008). Cry proteins are ingested by insects in the form of parasporal crystalline inclusions. In the alkaline environment of the insect midgut, these crystals are solubilized and hydrolyzed into protoxins, which subsequently bind to specific receptors on the midgut epithelial cells.

This binding triggers pore formation, disrupts osmotic balance, and ultimately leads to cell death (Fig. 1). The widespread destruction of midgut epithelial cells compromises the integrity of the gut lining, allowing Bt and other gut bacteria to invade the nutrient-rich hemocoel, proliferate, and cause septicemia, resulting in insect mortality (Raymond *et al*. 2010). Due to its efficacy, Bt-based products containing spores and crystalline toxin mixtures have been commercialized for controlling various insect larvae, including caterpillars, beetles, and mosquitoes. Additionally, transgenic crops expressing Bt toxin genes, such as cotton and maize, have been cultivated in many countries. These genetically modified (GM) crops have been widely adopted globally, effectively reducing insect damage and minimizing the need for chemical pesticides (Raymond *et al*. 2010).

Evolution of cry protein classification

Cry proteins exhibit high specificity toward different insect species, primarily due to their selective binding to surface proteins on the microvilli of midgut epithelial cells in insect larvae. This binding specificity determines their insecticidal activity. Initially, Cry proteins were classified based on their target insect groups. Among them, Cry1, Cry2, and Cry9 exhibit toxicity against Lepidoptera. Cry2 also targets both Lepidoptera and Diptera. Cry3, Cry7, Cry8, and Cry1Ia are effective against Coleoptera. Cry4, Cry10, Cry11, and Cyt exhibit toxicity toward Diptera. Cry5, App6, and Cry12 are toxic to nematodes and the two-spotted spider mite (Krieg *et al*. 1983; Höfte & Whiteley 1989; Haffani *et al*. 2001; Wei *et al*. 2003; Ruiz de Escudero *et al*. 2007; Hernández-Martínez *et al*. 2008; Hernández-Soto *et al*. 2009; Domínguez-Arrizabalaga *et al*. 2020; Valtierra-de-Luis *et al*. 2020; Guo *et al*. 2022; Unzue *et al*. 2022).

As research on Cry proteins advanced and became increasingly complex, a revised classification system was introduced in 1998, categorizing proteins solely based on amino acid sequence similarity (Crickmore *et al*. 1998). This

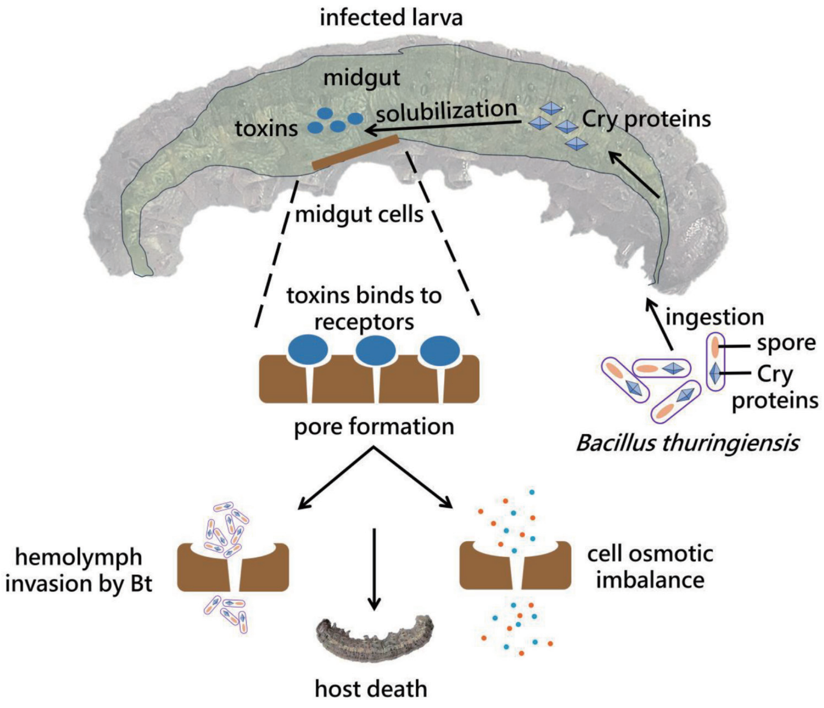

Fig. 1. Mode of action of *Bacillus thuringiensis* in Lepidoptera.

four-tiered nomenclature system groups proteins with more than 45% sequence similarity into the same primary classification (e.g., Cry1, Cry2). Proteins with less than 78% similarity within a primary group are further assigned to different secondary levels (e.g., Cry1A, Cry1B). The tertiary level is designated for proteins within a secondary group that share less than 95% sequence similarity (e.g., Cry1Aa, Cry1Ab). Finally, the quaternary level differentiates proteins within a tertiary classification that exhibit over 95% sequence identity (e.g., Cry1Aa1, Cry1Aa2). Beyond Cry proteins, which are insecticidal toxins derived from crystalline inclusions, the classification also includes Cyt proteins, which exhibit *in vitro* cytolytic activity and are specifically toxic to dipteran insects, as well as Vip proteins, which are secreted by Bt during the vegetative growth phase (Estruch *et al*. 1996).

As further research expanded the discovery of toxic proteins from Bt and other bacterial species, the nomenclature system was updated to classify proteins based on full-length sequence alignment, with adjusted similarity thresholds of 45%, 76%, and 95%. This revision led to the definition of sixteen structural classes of pesticidal proteins, including crystalline and insecticidal (Cry) proteins, cytolytic proteins (Cyt), Vip, proteins related to the ADP-ribosyltransferase active component of binary toxins (Vpa), proteins related to the binding component of binary toxins (Vpb), beta pore-forming pesticidal proteins (Tpp and Mpp), aegerolysin like pesticidal proteins (Gpp), alpha helical pesticidal proteins (App), sphaericolysin like pesticidal proteins (Spp), proteins related to the "Makes Caterpillars Floppy" toxins (Mcf), proteins related to the Mtx1 toxin (Mtx), proteins related to the *Photorhabdus* insect-related toxin (Pra and Pra), pesticidal proteins that are part of the Membrane Attack Complex/Perforin superfamily (Mpf), and a holding class for pesticidal proteins with currently uncharacterized structures (Xpp) (Crickmore *et al*. 2021). This refined classification system continues to facilitate the systematic identification and characterization of novel pesticidal proteins, contributing to advancements in biocontrol strategies.

Subspecies of *B*. *thuringiensis* and their insecticidal specificity

Bt is classified into approximately 30 subspecies based on flagellar antigenic properties and various biochemical tests. These subspecies exhibit different insecticidal activities against specific insect orders. For example, Bt subsp. *aizawai* (Bta) and Bt subsp. *kurstaki* (Btk) are highly toxic to lepidopteran pests such as *Plutella xylostella* (Linnaeus, 1767) and *Agrotis ipsilon* (Hufnagel, 1766) (Iqbal *et al*. 1996; Mashtoly *et al*. 2011). Bt subsp. *san diego* and Bt subsp. *tenebrionis* are effective against coleopteran insects, while Bt subsp. *israelensis* (Bti) has high toxicity against dipteran pests, including mosquitoes (McPherson *et al*. 1988; Bauer 1990; Ben-Dov 2014).

Advantages and challenges of *B*. *thuringiensis* applications

Bt-based biopesticides offer several advantages, including safety for humans and non-target organisms, reduced pesticide residues in food, conservation of natural enemies, and promotion of biodiversity in managed ecosystems. However, their application faces challenges such as the potential development of insect resistance and environmental factors affecting Bt activity. To address these limitations, researchers are actively developing novel Bt strains with enhanced insecticidal activity and integrating Bt with other biocontrol strategies, including entomopathogenic fungi (EPFs), viruses, and nematodes, to improve pest management efficacy.

The first commercial Bt cotton was introduced in the United States in 1996, effectively controlling three major lepidopteran pests: *Heliothis virescens* Fabricius, 1777, *Helicoverpa zea* (Boddie, 1850), and *Pectinophora gossypiella* (Saunders, 1844) (Mendelsohn *et al*. 2003). Subsequently, GM crops expressing Bt proteins, such as maize, soybean, and eggplant, have

been widely adopted, with their cultivation areas expanding annually (Meissle *et al*. 2022).

Effects of Bt crops on non-target organisms

The potential impact of Bt crops on non-target insects has been a major concern. A laboratory-scale study first reported that monarch butterfly (*Danaus plexippus* (Linnaeus, 1758)) larvae experienced mortality after exposure to Bt maize pollen, raising concerns about the potential risks of Bt toxins to non-target species (Losey *et al*. 1999). However, further research has demonstrated that the insecticidal proteins used in commercial GM crops (Cry and Vip) do not pose harmful effects on pollinators such as honey bees (*Apis mellifera* Linnaeus, 1758) (Malone & Burgess 2009). A study comparing two Bt maize varieties—one expressing a single Bt protein and another expressing three Bt proteins- has found no adverse effects on honey bee larval survival or prepupal weight (Hendriksma *et al*. 2011). A meta-analysis summarizing data from 12 bibliographic databases, 17 specialized websites, 78 review articles, and over 7,200 records from 233 experiments and 120 studies concluded that Bt maize had minimal impact on non-target invertebrate communities in maize fields (Meissle *et al*. 2022).

Other bacterial pathogens for agricultural pest control

Among bacterial pathogens used in agricultural pest management, *Paenibacillus popilliae* (Dutky 1940) Pettersson *et al*. 1999 (formerly *Bacillus popilliae*) was the first microbial agent registered as an insecticide. It is primarily used to control the Japanese beetle (*Popillia japonica* Newman, 1838) in turfgrass (Adams & Wheeler 1946; Arthurs & Dara 2019). Additionally, *Chromobacterium subtsugae* Martin *et al*. 2007 and *Burkholderia* spp. have a broad host range and have been developed as broad-spectrum insecticides and miticides, effective against whiteflies, aphids, scale insects, and spider mites (Shannag & Capinera 2018; Golec *et al*. 2020; Shannag 2025).

Bacteria used in agricultural pest control are often isolated from environmental sources such as soil and plants. However, this traditional screening approach is time-consuming, labor-intensive, and has low efficiency. Examples of bacterial biopesticides obtained through this method include *B. thuringiensis*, *C. subtsugae*, *Burkholderia* spp., and *Pseudomonas putida* (Trevisan, 1889) Migula, 1895 (Starnes *et al*. 1993; Martin *et al*. 2007; Aksoy *et al*. 2008). In recent years, some studies have focused on isolating bacterial pathogens directly from infected insects or mites. For example, *Bacillus velezensis* Ruiz-García *et al*. 2005 w1 was isolated from diseased *Tetranychus urticae* Koch, 1836 (Li *et al*. 2019). Another notable example is *Photorhabdus luminescens* (Thomas and Poinar, 1979) Boemare *et al*. 1993, which was isolated from the gut of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar, 1976. This bacterium exists in a symbiotic relationship with the nematode, aiding in host infection and insect mortality (Boemare *et al*. 1993; Hsieh *et al*. 2023).

Synergistic effects of pathogenic micro-organisms

The combination of pathogenic micro-organisms with complementary modes of action can enhance insecticidal efficacy and delay the development of pest resistance. Recent studies have demonstrated notable synergistic effects among bacteria, fungi, and viruses used in pest management. For instance, *P. luminescens* (Pl) combined with Btk exhibited strong synergism against *Spodoptera frugiperda* (Smith, 1797), reducing the Bt dosage required for comparable mortality by up to fivefold. Higher proportions of Bt led to increased *P. luminescens* loads in the hemocoel and more severe midgut damage, indicating that Bt promotes *P. luminescens* invasion and augments its virulence (Chang *et al*. 2024).

Similarly, synergistic interactions between NPVs and Bt have been reported against *A. ipsilon*. *Mamestra brassicae* (Linnaeus, 1758)

multiple nucleopolyhedrovirus (MbMNPV) showed the highest virulence, while combinations of *Spodoptera litura* (Fabricius, 1775) MNPV (SlMNPV) and Bt produced synergistic effects, resulting in elevated larval mortality and altered survival dynamics compared to single-pathogen treatments. In contrast, other NPV–Bt combinations exhibited additive or variable interactions depending on strain and concentration (Mao *et al*. 2025).

Collectively, these findings highlight that inter-microbial synergy- whether between bacteria and viruses or among bacterial species- can substantially enhance biocontrol performance. Understanding these interactions provides a promising foundation for developing optimized microbial formulations that improve field efficacy while supporting sustainable pest management.

Future research directions in bacteria-based pest control

The success of microbial pest control strategies depends on their adaptability to environmental conditions, formulation stability, and synergy with other pest management measures. Future research should focus on: (1) Enhancing the virulence and speed of action of pathogenic strains to improve their effectiveness against target pests. (2) Improving the adaptability of microbial pathogens to extreme environmental conditions, such as low temperatures and arid climates, to expand their application range. (3) Increasing production efficiency for large-scale applications, ensuring the economic feasibility and sustainable supply of microbial pesticides. (4) Developing improved formulations to enhance ease of use, environmental persistence, and shelf life. (5) Exploring how insect-pathogenic bacteria can be effectively integrated into IPM systems, including their interactions with environmental factors and other IPM components. (6) Conducting more precise assessments of the environmental benefits of insect-pathogenic bacteria to promote their sustainable development and application. (7) Encouraging broader acceptance and adoption of microbial pesticides among farmers and the general public, increasing their market penetration.

In summary, bacteria-based microbial pesticides play a crucial role in agricultural pest control. However, their application requires careful consideration of multiple factors to achieve optimal pest management outcomes. Furthermore, certain insect-pathogenic bacteria, such as Bt, not only serve as agricultural insecticides but also produce a variety of bioactive compounds with diverse applications (Salehi Jouzani *et al*. 2025). These include plant disease suppression, plant growth promotion, biofuel production, bioplastic synthesis, nanoparticle fabrication, food preservation, cancer treatment, and heavy metal bioremediation (Salehi Jouzani *et al*. 2025). These multifunctional properties underscore the value of bacterial biopesticides not only in pest control but also as essential resources in sustainable agriculture and biotechnology.

FUNGI IN AGRICULTURAL PEST BIOCONTROL

As of 2023, a total of 27 species of EPFs have been registered as biopesticides worldwide (Jiang & Wang 2023). Among these, the most commonly applied EPFs in agricultural pest control belong to the genera *Beauveria*, *Metarhizium*, *Cordyceps*, *Hirsutella*, *Verticillium*, *Lecanicillium*, and *Paecilomyces*. These fungi not only serve as effective agents for pest control but, in some cases, can also act as endophytic insect pathogenic fungi (EIPFs). EIPFs colonize plant tissues, where they promote plant growth and enhance the plant's immune system, contributing to improved plant health (Kabaluk & Ericsson 2007; Gupta *et al*. 2022).

Among these genera, *Beauveria* spp. is the most widely used for pest management due to its ability to produce more than ten insecticidal metabolites, making it a valuable tool for controlling a broad range of pests (Zhang *et al*. 2020; Xing *et al*. 2024). *Metarhizium* spp., commonly found in soil, plant roots, and insects, exhibit a broad host range and are a key model

for studying the co-evolution of fungal virulence in insects and plants (Sheng *et al*. 2022). During the later stages of infection, insects infected with *Metarhizium* sp. show the presence of destruxin, an insecticidal metabolite produced by the fungus (Suzuki *et al*. 1970; Liu & Tzeng 2012). *Cordyceps* fungi, which are parasitic on insects or arthropods, are effective in controlling pests such as whiteflies, aphids, banana root borers (*Cosmopolites sordidus* (Germar, 1823)), and spider mites (Negrete González *et al*. 2018; Nishi *et al*. 2023; Wang *et al*. 2023). During their invasion of insect hosts, *Cordyceps* fungi secrete various enzymes and small insecticidal toxins that contribute to their pest control capabilities (Wang *et al*. 2023; Xing *et al*. 2024). Below is a detailed description of the more commonly applied species in pest control.

Beauveria bassiana

Beauveria bassiana (Bals.-Criv.) Vuill., 1912, an insect pathogenic fungus, is classified within the phylum Ascomycota, class Sordariomycetes, order Hypocreales, family Cordycipitaceae, and the genus *Beauveria*. *B. bassiana* is a widely applied biopesticide in agricultural pest control, capable of infecting over 1,000 insect species and exhibiting toxic effects against a broad range of agricultural pests (Araújo & Hughes 2016; Vivekanandhan *et al*. 2024). Notably, it is relatively safe for non-target organisms and vertebrates (Zimmermann 2007). In addition to its use as a biocontrol agent, *B. bassiana* can also function as an endophytic fungus, colonizing plant tissues without causing symptoms, and even promoting host plant growth (Lopez & Sword 2015; McKinnon *et al*. 2017; Afandhi *et al*. 2019; Grabka *et al*. 2022; Liu *et al*. 2022).

Infection process and mechanisms of *B. bassiana*

Upon infecting insects, *B. bassiana* attaches its conidia (asexual spores) to the insect cuticle, where the conidia germinate and form an appressorium. This structure combines biochemical and mechanical pressures to penetrate the cuticle and enter the insect's hemocoel, where the fungus produces mycelium that invades the insect's tissues. The fungus then generates blastospores, eventually leading to the host's loss of mobility and death. During this process, mycelium grows from the insect's intersegmental membranes, eventually covering the insect's body and producing a mass of white, powdery spores. These spores can spread to other healthy insects, continuing the cycle of infection (Fig. 2). *B. bassiana* infections rely on multiple mechanisms, including the production of a variety of toxic secondary metabolites. These metabolites enhance fungal invasion and suppress the insect's immune defense system (Pedrini 2022). More than ten insecticidal metabolites have been identified, including nonribosomal peptides and polyketides such as beauvericin, bassianolide (cyclooligomeric nonribosomal peptides), beauverolides (cyclic peptides), oosporein (dibenzoquinone), bassiatin (diketomorpholine), and tenellin (2-pyridone) (Zhang *et al*. 2020). While the exact roles of these secondary metabolites remain unclear, they are generally correlated with the virulence of fungal strains (Butt *et al*. 2016; Zhang *et al*. 2020; Pedrini 2022). For example, Vivekanandhan *et al*. (2024) isolated secondary metabolites produced by *B. bassiana* and evaluated their toxicity against *Tuta absoluta* (Meyrick, 1917) larvae, and found that 9,10-octadecadienoic acid exhibited the strongest toxicity against the larvae. In a study by Tsai *et al*. (2006), the pathogenicity of eight local Taiwanese *B. bassiana* strains was compared, revealing significant differences in their effectiveness against pests such as the diamondback moth (*P. xylostella*) and beet armyworm (*Spodoptera exigua* (Hübner, 1808)).

Endophytic characteristics and plant growth promotion

The endophytic nature of *B. bassiana* enables it to colonize plant tissues, promote plant growth, and improve plant health without causing harm. For example, it has been shown to enhance the antioxidant activity of lettuce

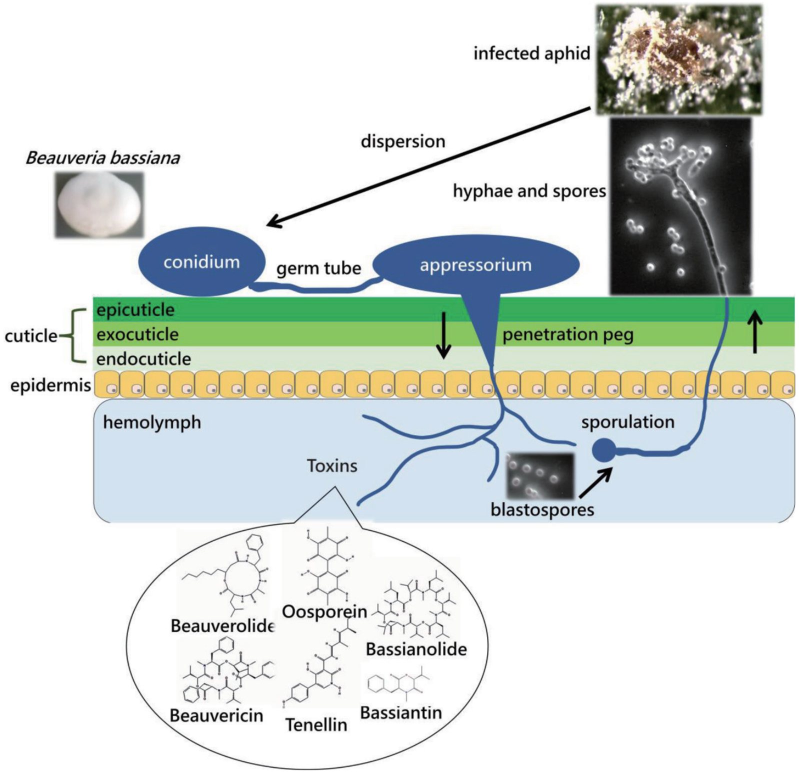

Fig. 2. Mode of action of *Beauveria bassiana* in aphids.

(*Lactuca sativa* L.) and can even be transmitted vertically through seeds in crops like cotton (*Gossypium hirsutum* L.) and common bean (*Phaseolus vulgaris* L.) (Quesada-Moraga *et al.* 2014; Lopez & Sword 2015; Afandhi *et al.* 2019; Macuphe *et al.* 2021). These characteristics open new avenues for IPM strategies, making *B. bassiana* a key player in sustainable agriculture. Continued research into its endophytic traits will be essential in developing innovative pest management strategies that align with ecological principles.

Challenges and future directions

B. bassiana, as a biocontrol agent, can be integrated with other pest management strategies in the integrated management of agricultural pests. For instance, in the control of the coffee berry borer (*Hypothenemus hampei* (Ferrari, 1867)), the combination of *B. bassiana* and CMU-C1 lure-modified traps (Krutmuang *et al.* 2023) has been shown to reduce pest populations in coffee plantations. Different strains of *B. bassiana* exhibit variations in growth characteristics, virulence, and environmental adaptability, necessitating the selection of suitable strains based on the target pest and environmental conditions. However, large-scale application of *B. bassiana* still faces challenges related to inconsistent efficacy. Future research should focus on enhancing its endophytic potential, improving formulation development, and optimizing application techniques to strengthen its role in IPM.

Metarhizium anisopliae

The genus *Metarhizium*, commonly known as EPFs, belongs to the phylum Ascomycota, class Sordariomycetes, order Hypocreales, and family Clavicipitaceae. Initially, the genus contained only four species; however, advancements in DNA sequencing techniques

have expanded its classification to include over 30 species (Bischoff *et al*. 2009; Brunner-Mendoza *et al*. 2019). The host range of different *Metarhizium* species varies significantly. For example, *Metarhizium anisopliae* (Metschn.) Sorokīn, 1883 has a broad host range, infecting more than 200 insect species, while *M. acridum* is highly specialized, primarily targeting Orthoptera (Gao *et al*. 2011; Brunner-Mendoza *et al*. 2019). Some species within this genus exist as saprophytic fungi in the soil, serving as rhizosphere microbes, while others function as endophytic fungi colonizing plant tissues or as potent insect pathogens (Hu & Leger 2002; Wyrebek *et al*. 2011; Steinwender *et al*. 2015; Moonjely & Bidochka 2019).

M. anisopliae: A model species

M. anisopliae is the type species of the *Metarhizium* genus and is a well-known entomopathogenic fungus with a broad host range, infecting over 200 insect species, including many agricultural pests (Lomer *et al*. 1997; Gao *et al*. 2011; Contreras *et al*. 2014; Gabarty *et al*. 2014; Perumal *et al*. 2024). It also exhibits endophytic behavior, colonizing plant tissues and promoting plant growth (González-Pérez *et al*. 2022; Liu *et al*. 2022). The species was initially isolated from the beetle *Anisopliae austriaca* (Herbst, 1783) and was originally named *Entomophthora anisopliae*. However, subsequent studies based on conidial morphology and DNA sequence data led to its reclassification as *M. anisopliae* (Brunner-Mendoza *et al*. 2019).

Infection process of *M. anisopliae*

The infection process of *M. anisopliae* closely resembles that of *B. bassiana* and consists of six key stages: (1) Attachment of Conidia: The fungal conidia adhere to the insect cuticle. (2) Germination and Growth: The conidia germinate and develop. (3) Appressorium Formation: A specialized infection structure called the appressorium is formed. (4) Cuticle Penetration: The fungus penetrates the insect cuticle and invades the hemocoel. (5) Fungal Proliferation: Mycelium spreads throughout the insect's tissues, producing blastospores, leading to host immobilization and eventual death. (6) Sporulation and Transmission: Mycelium emerges from the insect's intersegmental membranes, covering the body with powdery white spores, which are then disseminated to infect new hosts (Aw & Hue 2017; Peng *et al*. 2022).

Molecular mechanisms underlying infection

Early-stage attachment and conidial germination

During the initial infection stage, *M. anisopliae* conidia attach to the insect cuticle. The conidial surface contains hydrophobic proteins that facilitate adhesion to the host's hydrophobic cuticle (Tseng *et al*. 2014). Once attached, cuticular lipids trigger conidial germination, with carbon and nitrogen sources serving as essential elements for spore activation (Ment *et al*. 2010).

Appressorium formation and cuticle penetration

The development of the appressorium is crucial for host infection. The *ODC1* gene encodes ornithine decarboxylase, which is involved in conidial germination and appressorium formation, while the *Mpl1* gene encodes MPL1, which regulates lipid homeostasis and appressorium differentiation (Wang & Leger 2007b; Pulido *et al*. 2011). Protein kinase A (PKA) plays a role in appressorium and penetration peg differentiation by regulating glycerol permeability under hypoosmotic conditions, thereby maintaining turgor pressure within the appressorium (Fang *et al*. 2009). The *Mos1* gene encodes an osmosensor, which aids in osmoregulation (Wang *et al*. 2008). The high turgor pressure generated enables the fungus to apply mechanical force, facilitating cuticle penetration (Lovett & Leger 2015). In addition, the conidial appressorium secretes the MAD1 protein, which binds to the host insect cuticle (Wang & Leger 2007a).

Enzymatic degradation of the cuticle

The penetration process of EPFs involves the action of multiple hydrolytic enzymes that degrade the insect cuticle. These enzymes include elastase, subtilisins (Pr1 and Pr2), trypsins, chymotrypsins, carboxypeptidases, chitinases, and lipolytic enzymes (Joshi & Leger 1999; Bagga *et al*. 2004; Beys da Silva *et al*. 2010; Santi *et al*. 2010a, 2010b; Schrank & Vainstein 2010; Aw & Hue 2017). The expression of these enzymes varies depending on the host species. For instance, different isoforms of elastase (Pr1A–K) may be involved in the early infection stages of *M. anisopliae* and play a role in host recognition. Notably, the Pr1I protease is expressed during the infection of the cotton stainer bug (*Dysdercus peruvianus* Guérin-Méneville, 1831) (Santi *et al*. 2010b). In contrast, Pr1J is significantly upregulated in infections of the cockroach (*Blaberus giganteus* (Linnaeus, 1758)) and the gypsy moth (also called spongy moth) (*Lymantria dispar* (Linnaeus, 1758)) but not in the Japanese beetle (*P. japonica*) (Freimoser *et al*. 2005). These variations in enzyme expression are associated with *M. anisopliae*'s ability to recognize specific hosts.

In culture media simulating infections of *D. peruvianus* and cattle tick (*Rhipicephalus microplus* (Canestrini, 1888)) by *M. anisopliae*, chitinases were detected. Although these chitinases shared the same molecular weight, they exhibited differences in isoelectric points (pI), suggesting that *M. anisopliae* may secrete distinct chitinase isoforms depending on the host species (Santi *et al*. 2010b). Furthermore, mutant strains of *M. anisopliae* lacking chitinase genes exhibited significantly lower virulence against *D. peruvianus*, highlighting the crucial role of chitinase in fungal pathogenesis (Boldo *et al*. 2009).

Destruxins: Key secondary metabolites of *M. anisopliae*

The most common secondary metabolites found in the fermentation broth of *M. anisopliae* are a family of cyclic peptide toxins known as destruxins. These compounds are cyclic hexadepsipeptides composed of an α-hydroxy acid and five amino acid residues (Hsiao & Ko 2001; Pedras *et al*. 2002; Wang *et al*. 2012). Destruxins are classified into five main groups (A to E) based on their chemical structures (Pedras *et al*. 2002). As of 2010, a total of 38 different destruxins had been identified, with most of them being secondary metabolites of *M. anisopliae* (Schrank & Vainstein 2010). Among these, destruxins A, B, and E exhibit significant insecticidal activity (Sree *et al*. 2008). Destruxin B was the first to be synthesized through organic synthesis (Kuyama & Tamura 1965). The biosynthesis of destruxins was first identified in *M. anisopliae* and is regulated by a non-ribosomal peptide synthetase (NRPS) gene cluster (Wang *et al*. 2012). Destruxins exert their toxic effects by opening cellular Ca^{2+} channels, disrupting the Ca^{2+}/calmodulin signaling pathway. This process leads to an influx of extracellular Ca^{2+}, causing insect muscle tetanic contraction, paralysis, and ultimately death. Additionally, destruxins suppress the insect immune response, thereby enhancing fungal virulence (Ruiz-Sanchez *et al*. 2010; Wang *et al*. 2012).

Conclusion

Metarhizium fungi hold significant application value in the fields of entomopathology and biological control. The genus is diverse, ranging from the broad host-range *M. anisopliae* to the host-specific *M. acridum*, demonstrating different ecological adaptation strategies. Additionally, these fungi are not only efficient insect pathogens but also possess potential as endophytes and plant growth promoters, making them multifunctional biocontrol agents. Their infection mechanism involves attachment, spore germination, formation of infection structures, enzymatic degradation, and the toxic effects of secondary metabolites such as destruxins, illustrating a complex and precise pathogenic process. However, despite extensive research revealing their biological characteristics, *Metarhizium* still faces numerous challenges in practical applications. With advancements

in genomics, synthetic biology, and ecology, future research will help optimize the application potential of *Metarhizium*, promoting its practical use in sustainable agriculture and IPM.

Cordyceps javanica

The entomopathogenic fungus *Cordyceps javanica* (Bally) Kepler, Shrestha & Spatafora, 2017 belongs to the phylum Ascomycota, class Sordariomycetes, order Hypocreales, family Cordycipitaceae, and genus *Cordyceps*. The genus *Cordyceps* comprises 629 known species, all of which parasitize insects or other arthropods (Qu *et al*. 2022). Among them, several species are commonly used in biopesticides, including *C. javanica*, *Cordyceps fumosorosea* (Wize) Kepler, Shrestha & Spatafora, 2017, *Cordyceps farinosa* (Holmsk.) Kepler, Shrestha & Spatafora, 2017, and *Cordyceps cateniannulata* (Z.Q. Liang) Kepler, Shrestha & Spatafora, 2017 (Jiang & Wang 2023). Notably, *C. javanica* has the most extensive genomic data available among these species (https://www.mycobank.org).

Infection process of *C. javanica* in *Acyrthosiphon pisum*

The infection process of *C. javanica* in the pea aphid (*Acyrthosiphon pisum* (Harris, 1776)) follows a well-defined sequence of events. After *C. javanica* conidia adhere to the insect cuticle, they germinate and form germ tubes and penetration pegs. Mucilage matrices can be observed at the interface between the germ tubes and the cuticle. The germ tubes develop into hyphae, with some penetrating the cuticle and others accumulating on the exocuticle. As hyphal proliferation continues, additional mucilage matrices are secreted near the attachment structures, facilitating the dissolution and erosion of the insect's cuticle.

Once inside, the hyphae breach the basal membrane and enter the hemocoel, where they proliferate throughout the host body. On the cuticle surface, the fungus differentiates into conidiophores, producing chains of colorless conidia that serve as secondary infection propagules. Eventually, mycelial outgrowths emerge from the aphid cadaver, enabling further dispersal. Proteolytic enzymes, such as Pr1 protease and chitinase, have been detected in culture media containing *C. javanica* conidia, indicating their involvement in host penetration and infection (Wang *et al*. 2023).

Insecticidal metabolites in *C. javanica* fermentation broth

Beyond enzymatic virulence factors, *C. javanica* produces insecticidal secondary metabolites, including small-molecule toxins such as heteratisine. Heteratisine is an alkaloid that binds to the peripheral anionic site of acetylcholinesterase, thereby inhibiting the enzyme's activity in aphids. This mode of action disrupts neurotransmission and contributes to the insecticidal effect of *C. javanica* fermentation broth (Xing *et al*. 2024).

Pathogenicity of *C. javanica* against various insect pests

Several studies have demonstrated the efficacy of *C. javanica* against a range of insect pests. *C. javanica* has shown significant pathogenicity against the Asian citrus psyllid (*Diaphorina citri* Kuwayama, 1908), a major vector of citrus greening disease (Meyer *et al*. 2008; Ou *et al*. 2019b; Awan *et al*. 2021). Additionally, Nguyen *et al*. (2017) isolated *C. javanica* strains highly virulent against the diamondback moth (*P. xylostella*) and the common cutworm (*S. litura*).

Compatibility of *C. javanica* with natural enemies in pest control

When applying *C. javanica* for field pest management, its impact on natural enemies must be carefully evaluated. Ou *et al*. (2019a) analyzed the pathogenicity of *C. javanica* against *Eretmocerus hayati* Zolnerowich & Rose, 1998, a parasitoid of the whitefly *Bemisia tabaci* (Gennadius, 1889). Their findings revealed that higher concentrations of *C. javanica* increased pathogenicity against *E. hayati*, but its impact

on the parasitoid was significantly lower than on *B. tabaci*. According to the International Organization for Biological Control (IOBC) standards, *C. javanica* was harmless to *E. hayati* pupae and only slightly harmful to adults. Interestingly, infection of *B. tabaci* nymphs with *C. javanica* extended the lifespan and developmental period of *E. hayati* offspring.

Semi-field trials demonstrated that the combined application of *C. javanica* and *E. hayati* resulted in a superior control of *B. tabaci* compared to either agent alone. These findings highlight the potential of integrating *C. javanica* with parasitoids for enhanced and compatible biological control strategies against *B. tabaci*.

ENTOMOPATHOGENIC VIRUSES

Baculoviridae: A major group of entomopathogenic viruses

The Baculoviridae family is widely used for insect pest control and primarily infects insects of the orders Lepidoptera, Hymenoptera, and Diptera. These large, double-stranded circular DNA viruses range from 80 to 180 kbp in size and contain 90 to 180 genes. Baculoviruses are characterized by their rod-shaped nucleocapsids enclosed within a lipid envelope and occlusion bodies (OBs) measuring 0.15−5 μm (Okano *et al*. 2006; Harrison *et al*. 2018; Sajid *et al*. 2021).

Classification based on occlusion bodymorphology

Baculoviruses are classified into two major types based on their OB structures: nucleopolyhedroviruses (NPVs) and granuloviruses (GVs). NPVs produce large OBs in the nuclei of infected cells, encapsulating multiple rod-shaped virions within a crystalline polyhedral protein matrix. These OBs, also known as polyhedral inclusion bodies, are smooth-surfaced. In contrast, GVs form small, granular OBs composed of granulin protein, each typically containing a single virion. GVs are only found in Lepidoptera (Winstanley & O’Reilly 1999; Williams *et al*. 2022). The virions within OBs are referred to as occlusion-derived viruses (ODVs), which consist of one or more rod-shaped nucleocapsids with unique structural polarity, enclosed within a membrane envelope (Harrison *et al*. 2018).

Baculovirus infection cycle

The infection cycle of baculoviruses produces two forms of virions: ODVs and budded viruses (BVs). Upon ingestion by a susceptible insect, the high alkalinity and proteolytic enzymes in the midgut dissolve the OBs, releasing ODVs. These ODVs penetrate the peritrophic membrane and fuse with the midgut epithelium cell membranes through interactions involving a multiprotein complex of per os infectivity factors (PIFs). The nucleocapsids enter the midgut epithelial cells, disrupting tissue integrity and ultimately liquefying the host (Fig. 3). (Kawanishi *et al*. 1972; Horton & Burand 1993; Javed *et al*. 2017; Erlandson *et al*. 2019; Wang *et al*. 2019).

BVs are produced when nucleocapsids bud from the infected cell membrane, spreading through the hemolymph and tracheal system to infect other tissues (Passarelli 2011). BVs typically contain a single nucleocapsid enclosed within a cytoplasmic membrane-derived envelope, incorporating envelope fusion glycoproteins (EFPs). These glycoproteins form dense spike-like projections on one or both ends of the virion (Wang *et al*. 2016). In the later stages of infection, ODVs are assembled and enclosed within the nucleus to form OBs.

Host manipulation and virus dissemination

Baculoviruses manipulate host behavior to enhance viral dissemination. Infected larvae exhibit climbing behavior before death, positioning themselves at the upper parts of the host plant. The viral enzyme ecdysteroid UDP-glucosyltransferase (EGT) inhibits the activation of molting hormones, preventing the larvae from molting (O’Reilly & Miller 1989). The cuticle of infected larvae is weakened by viral proteases, such as cathepsin-like protease

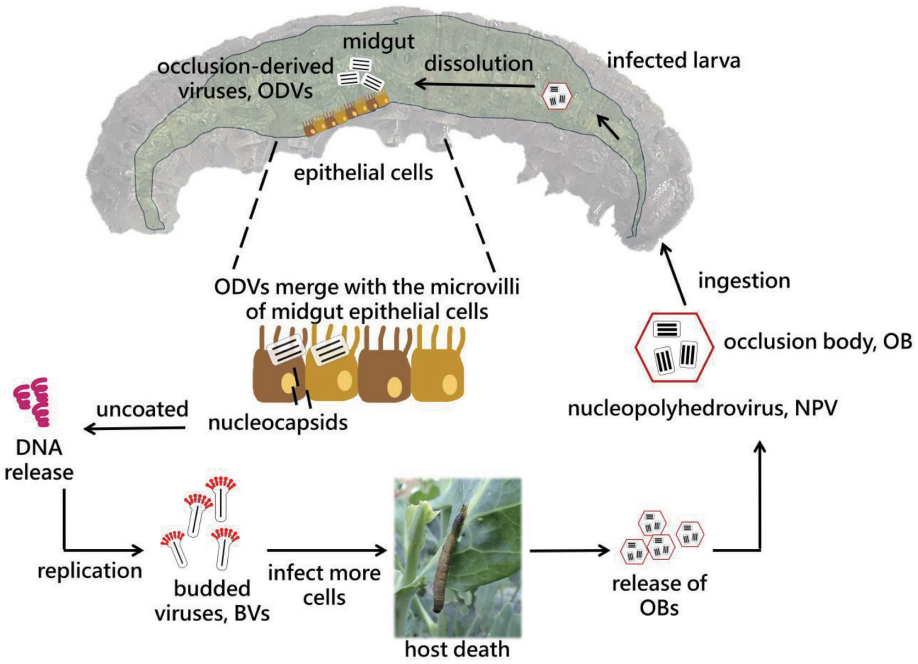

Fig. 3. Mode of action of nucleopolyhedroviruses in Lepidoptera.

and chitinase, leading to host liquefaction and the release of millions of OB progeny onto plant surfaces. This process ensures efficient viral transmission to new hosts (Hawtin *et al.* 1997).

Taxonomic classification and genetic characteristics of Baculoviridae

Baculoviridae is classified into four genera based on phylogenetic relationships, genomic characteristics, host range, and OB morphology: *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus*, and *Deltabaculovirus*. These four genera share a total of 38 homeotic genes, which are highly conserved and involved in essential functions such as DNA replication, late gene transcription, and virion structure (Jehle *et al.* 2006; Garavaglia *et al.* 2012; Javed *et al.* 2017).

Members of the genus *Alphabaculovirus* infect lepidopteran larvae, with polyhedral OBs containing multiple virions. The occlusion-derived virus (ODV) undergoes nucleocapsid envelopment within the nucleus. Members of the genus *Betabaculovirus*, isolated only from lepidopteran larvae, form granular OBs, and ODV nucleocapsid envelopment occurs in the nuclear-cytoplasmic milieu after the nuclear membrane is lost. Both *Gammabaculovirus* and *Deltabaculovirus* exhibit polyhedral OBs containing multiple virions (Harrison *et al.* 2018).

Transmission and environmental persistence of baculoviruses

Baculoviruses spread among insects through both horizontal and vertical transmission. Horizontal transmission, the primary mode, occurs when insects ingest contaminated food or come into contact with OBs on egg surfaces. Vertical transmission involves the transfer of viruses from adult insects to their offspring via infected eggs (Doane 1969). Once a suitable host is infected, the virus undergoes a developmental cycle lasting approximately 1 wk, ultimately causing host liquefaction and releasing OBs into the environment. These OBs represent a stable form of the virus, exhibiting high resistance

to chemical and physical degradation, as well as UV inactivation. Additionally, studies have documented the ability of baculoviruses to persist asymptomatically in host populations (Myers & Cory 2016).

Host specificity and application in pest control

Compared to other entomopathogens such as EPFs and *B. thuringiensis*, baculoviruses exhibit exceptionally high host specificity. This trait significantly reduces non-target effects and minimizes ecological disruption when applied in the field (Armenta *et al*. 2003; Williams *et al*. 2022). However, since infected insects continue feeding for several days to weeks before death, they may still cause agricultural damage. To mitigate this issue, high concentrations of OBs or scheduled OB applications can target pests at their earliest developmental stages, reducing damage (Harper 1973). Additionally, genetic modification of baculoviruses to express insecticidal protein genes has been explored to accelerate pest mortality, inhibit feeding, or enhance overall virulence (Kroemer *et al*. 2015).

Behavioral manipulation by baculoviruses

Baculoviruses are known to alter host behavior post-infection. For instance, *L. dispar* larvae infected with baculoviruses exhibit altered nocturnal behavior, climbing to the tops of plants before death. This behavior, mediated by the EGT gene, ensures that liquefied cadavers release viral particles from elevated positions, enhancing transmission efficiency (Hoover *et al*. 2011). Additionally, viral protein tyrosine phosphatase (PTP) expression has been linked to increased host locomotor activity, promoting wandering behavior and further facilitating viral dispersal (Katsuma *et al*. 2012; van Houte *et al*. 2012). Phylogenetic analyses of PTP and EGT genes suggest that these genes may have originated from lepidopteran hosts, indicating that baculoviruses may have acquired advantageous genes during co-evolution with their hosts (Clem & Passarelli 2013).

Development of baculovirus-based biopesticides in Taiwan

In Taiwan, NPVs were isolated from *S. litura* and *S. exigua* larvae (Tuan *et al*. 1994, 1995). These viruses were highly pathogenic, inducing nuclear hypertrophy and tissue disintegration, with larval susceptibility decreasing with age. However, large-scale application was constrained by high production costs, as the virus had to be propagated in noctuid larvae prior to extraction. Traditional production methods involved grinding NPV-infected larvae, followed by viral extraction (Tuan *et al*. 1998). Alternatively, infected larvae could be frozen and processed when needed, although cold storage was found to reduce viral activity.

Recent advancements have overcome formulation challenges, resulting in a water-dispersible granule formulation that remains stable at room temperature, thereby greatly enhancing its usability (https://www.acri.gov.tw/Uploads/Item/14bc92c3-c799-4a8b-94c2-3c74ecf7b99b.pdf). Additionally, automated mass production has been integrated with AI technology. This system combines image recognition with robotic handling to automate insect rearing, viral infection, disease monitoring, viral extraction, and storage, drastically reducing labor costs and enhancing production efficiency (Deng *et al*. 2024).

Together, these developments demonstrate Taiwan's steady progress toward industrial-scale production and practical application of baculovirus-based biopesticides for sustainable pest management.

CONCLUSIONS

Pathogenic microorganisms hold significant potential for agricultural pest management, offering environmentally friendly solutions that enhance biodiversity and reduce reliance on chemical pesticides. Bacterial pathogens such as *B. thuringiensis*, as well as EPFs like *B. bassiana*, *M. anisopliae*, and *C. javanica* have demonstrated remarkable efficacy in pest control. Among these,

Bt has been widely adopted in agriculture due to its insect-specific toxic proteins and minimal impact on non-target organisms, playing a crucial role in managing pests affecting crops such as cotton and maize.

However, the use of microbial control agents still faces notable challenges, including the evolution of insect resistance, environmental variability affecting efficacy, and limitations in formulation stability and shelf life. Addressing these issues requires continuous innovation in strain improvement, formulation technology, and the strategic integration of microbial agents with complementary pest management tactics. Future research should focus on enhancing the virulence, stability, and field adaptability of these agents, while deepening our understanding of their ecological interactions within IPM systems. Such efforts will be key to realizing the full potential of microbial biopesticides as sustainable tools for modern agriculture.

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農業重要害蟲防治用病原微生物研究與發展

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摘要

張淑貞、李中潔、江明耀。2026。農業重要害蟲防治用病原微生物研究與發展。台灣農業研究 75(1):27–49。

病原性微生物在農業害蟲防治中具關鍵地位，能透過寄生、感染或產生毒素有效抑制害蟲族群，為化學農藥提供環境友善且永續的替代方案，並推動病蟲害綜合管理 (integrated pest management; IPM)。昆蟲病原微生物的研究與應用歷史悠久，常見的昆蟲病原細菌如蘇力菌 (*Bacillus thuringiensis*)、病原真菌如白殭菌 (*Beauveria bassiana*)、黑殭菌 (*Metarhizium anisopliae*)、爪哇擬青黴菌 (*Cordyceps javanica*)，及昆蟲病原病毒如核多角體病毒 (Nucleopolyhedrovirus; NPV)。這些微生物因專一性高、對環境安全且可降低農藥依賴，已成為 IPM 中的重要組成。近年基因體學與生物技術的進展，促進了高效菌株的篩選與毒力機制解析。全基因體定序與轉錄體分析使有助於鑑定致病相關基因，並揭示毒素生成、寄主感染與適應機制。此外，特定酵素如幾丁質酶、蛋白酶及次級代謝物的研究，亦提升病原微生物的感染力與殺蟲效能，擴大其應用潛力。然而，微生物防治仍面臨環境適應性、寄主抗性及製劑穩定性等挑戰。整體而言，病原微生物防治技術已成為現代農業的重要方向，隨著生物技術與應用策略的持續進步，將有望成為永續害蟲管理與糧食安全的核心支柱。

關鍵詞：昆蟲病原微生物、微生物農藥、細菌、真菌、病毒。

投稿日期：2025 年 8 月 15 日；接受日期：2025 年 10 月 28 日。
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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):51–72 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0003

水稻耐旱機制與分子育種策略之進展

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摘要

林大鈞、林玉鈴、王怡雯、林思妤、李承彬、溫韋辰、王昭月、王強生、陳述。2026。水稻耐旱機制與分子育種策略之進展。台灣農業研究 75(1):51–72。

水稻為全球重要主糧作物，對水資源需求高，面對日益嚴峻的氣候變遷與水資源短缺挑戰，提升其耐旱能力已成為確保糧食安全與實現農業永續發展的關鍵課題。本文綜述水稻在乾旱逆境下於生理、生化及基因層級的調控機制，涵蓋滲透調節、活性氧清除、氣孔調控及根系結構等核心途徑。近年學者透過數量性狀基因座 (qualitative trait loci; QTLs) 定位與候選基因選殖，已鑑定多個與耐旱性相關之關鍵基因與區域，包括 qDTYs、$qDTR_8$、$qDLR_{8.1}$、*OsNAC5*、*OsCPK9* 及 *LEA* 蛋白基因等，為分子育種提供重要遺傳資源。在小分子調控方面，已鑑定逾 30 種與乾旱反應相關的 miRNA，其中 19 種於植物乾旱反應期間，具有明確的調控功能，如 miR393、miR398、miR160、miR167 及 DST-amiRNA，分別參與生長素訊號、抗氧化能力及氣孔開閉等機制，構成一層關鍵的基因表現調控網絡。育種實務面，耐旱育種已逐步導入分子標記輔助選拔與 QTLs 堆疊 (pyramiding) 策略，成功育成多個具耐旱性狀之水稻品系。然而，乾旱外表型之田間表現與準確量化仍是主要的挑戰課題，限制了遺傳增益的進一步提升。未來應結合高通量表型體分析技術、基因體與表觀基因體資訊，整合環境數據進行乾旱反應之系統性解析，以建構多維度調控網絡與關鍵選拔指標，進而加速耐旱水稻品種之開發，以因應氣候變遷下的挑戰，並實現作物生產系統的智慧化與永續轉型。

關鍵詞：水稻、乾旱耐受性、數量性狀基因座、候選基因、分子育種。

前言

全球人口預估於2100年將超過100億 (Samir *et al*. 2024)。因此，『糧食安全』已是目前農業所面臨的最嚴峻挑戰 (Tiwari *et al*. 2024)。水稻是提供逾全球 1/3 人口的主要糧食作物，更是提供亞洲人 80% 日攝取熱量來源 (D’Odorico *et al*. 2014)。然而，水稻卻因為其較小根系統結構、較薄表皮蠟質及快速閉合之氣孔，導致其是一種乾旱敏感的作物 (Sahebi *et al*. 2018)。同時，受全球氣候變遷的影響，多數糧食作物生產區域面臨水資源的匱乏 (Liu *et al*. 2025)。雖然，臺灣年平均降雨量的長期趨勢未明顯減少，但全年各季節連續乾旱日數，卻因受氣候變遷的影響而變化 (Shanka *et al*. 2025)，造成近來乾旱災情不斷，尤其臺灣民間素有『三年一小旱、五年一大旱』的說法，在 2002–2020 年間，共發生 8 次旱災與 4 次大規模停灌休耕事件，對水稻產業的穩定生產構成重大挑戰 (Narvaez *et al*. 2022)。放眼亞洲地區，約有 340 萬公頃灌溉稻田與 80 萬公頃陸稻持續受到乾旱威脅，尤以近年受聖嬰現

投稿日期：2025 年 9 月 9 日；接受日期：2025 年 11 月 14 日。
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象影響，加劇乾旱面積持續擴大 (Singh *et al*. 2016; Hasan *et al*. 2025)。

在全球氣候變遷與水資源日益拮据的背景下，開發耐旱水稻品種已成為確保糧食安全與提升農業永續性的關鍵策略之一。儘管水稻耐旱育種早已展開，但受限於 2 大瓶頸：其一，缺乏具高耐旱潛力的種原資源；其二，尚未建立適用田間多樣環境的精準耐旱表型分析技術。過去，國際水稻研究所 (International Rice Research Institute; IRRI) 曾對數千份水稻種原進行篩選，卻僅有少數品種 (系) 具有明確的耐旱特性 (Singh *et al*. 2016)，顯示傳統評估方式可能難以解析耐旱性狀之複雜遺傳架構。實際上，水稻面對乾旱逆境時，在生理、生化乃至基因調控表現機制等層面上，展現多維度且複雜交互作用的機制，包括氣孔調節、滲透調節物質累積及抗氧化防禦系統啟動等 (Upadhyaya & Panda 2019; Gupta *et al*. 2020; Melandri *et al*. 2020; Ma *et al*. 2024)。如此多維度的調控網絡，使得水稻的耐旱特性恐難以由單一表型指標進行精準的篩選，進而導致育種家不易自田間篩選出具耐旱潛力基因型的品種 (系)。因此，建立整合生理、生化及分子標誌的耐旱性評估體系，將有助於精準選拔具育種價值的品種 (系)，並可加速耐旱關鍵選拔指標的開發與導入應用。爰此，本研究整合了水稻在形態、生理及分子層面的耐旱反應機制，並嘗試建立系統化的生理–分子關聯模型，以提供未來耐旱育種之理論依據。

水稻乾旱逆境之形態、生理及生化反應機制

水稻在乾旱逆境下，於形態外觀上常出現多種顯著變化：植株高度下降、分蘗數減少、葉片生命週期縮短、葉面積縮小、葉片捲曲 (leaf rolling; LR) 程度增加、根莖比提高及早熟現象加劇，最終導致整體生物量減少。研究指出，葉片機動細胞 (motor cells) 在失水後產生收縮，造成葉片向上捲曲；同時，葉片厚度、葉脈結構、維管束及束鞘細胞 (bundle sheath cells) 也發生明顯的形態變異 (Yan *et al*. 2020; Gunnula *et al*. 2022)。

於生理層級，乾旱會限制葉組織細胞的分裂與生長，誘導氣孔關閉，進而降低細胞內二氧化碳 (carbon oxidate; CO_2) 濃度，導致光合作用速率 (photosynthetic rate) 下降。伴隨著膨壓與滲透壓調控失衡及氧化逆境升高，也進一步抑制呼吸作用，最終削弱水分利用的效率。在生化層面，乾旱逆境會降低葉綠體基質中 1, 5-二磷酸核酮糖羧化酶/加氧酶 (ribulose-1,5-bisphosphate carboxylase/oxygenase; Rubisco) 酵素活性與淨光合作用能力，同時誘導一系列抗逆代謝物質的累積，包括脯氨酸 (proline)、多胺 (polyamines)、離層素 (abscisic acid; ABA) 及抗氧化酵素等，以協助植物維持細胞穩定性及防禦能力 (Oladosu *et al*. 2019)。整體而言，乾旱逆境會造成水稻光合產物的分配與轉運失衡，進而影響稻穗充實與穀粒形成，導致降低穀粒的重量與大小，甚至在嚴重條件下，會引發植株的死亡。

現行研究發現，植物乾旱抗性 (drought resistance) 可定義為有限水資源下仍能維持穩定產量的綜合能力。因此，性狀具有高度的複雜性，涉及多維度的形態、生理及代謝反應路徑。爰此，學者已將植物耐旱反應歸類為 3 大類型 (Kramp *et al*. 2022)，分別為：迴避 (avoidance)：指植物透過形態與生理調節，維持較高組織水勢，以避免細胞脫水。通常具備此機制的植物多具深根系、氣孔關閉敏感性高、LR 度高及葉片常綠且有較佳的呼吸效率；逃脫 (escape)：為植物在發生嚴重土壤缺水前，得快速完成生育週期的策略，其特徵包含：早開花、早熟、高葉氮水平、高光合作用能力及較佳的同化物再動員力等特性 (Liao *et al*. 2023)；耐性 (tolerance)：則是植物處於低量組織水狀態下的生存能力，包含：組織滲透調節能力、較高保護性的溶質、脯氨酸及耐旱逆境蛋白酶含量等，或較高的氣孔導度，或可維持一定的光合作用水平 (圖 1)。

上述分類機制不僅有助於釐清水稻耐旱的反應多樣性，更可作為耐旱性狀分子育種之重要指標基礎，進而促進耐旱基因型品種 (系) 篩選的重要參考。爰此，本節將進一步綜整水稻乾旱逆境時之外觀表型、生理及生化層面反

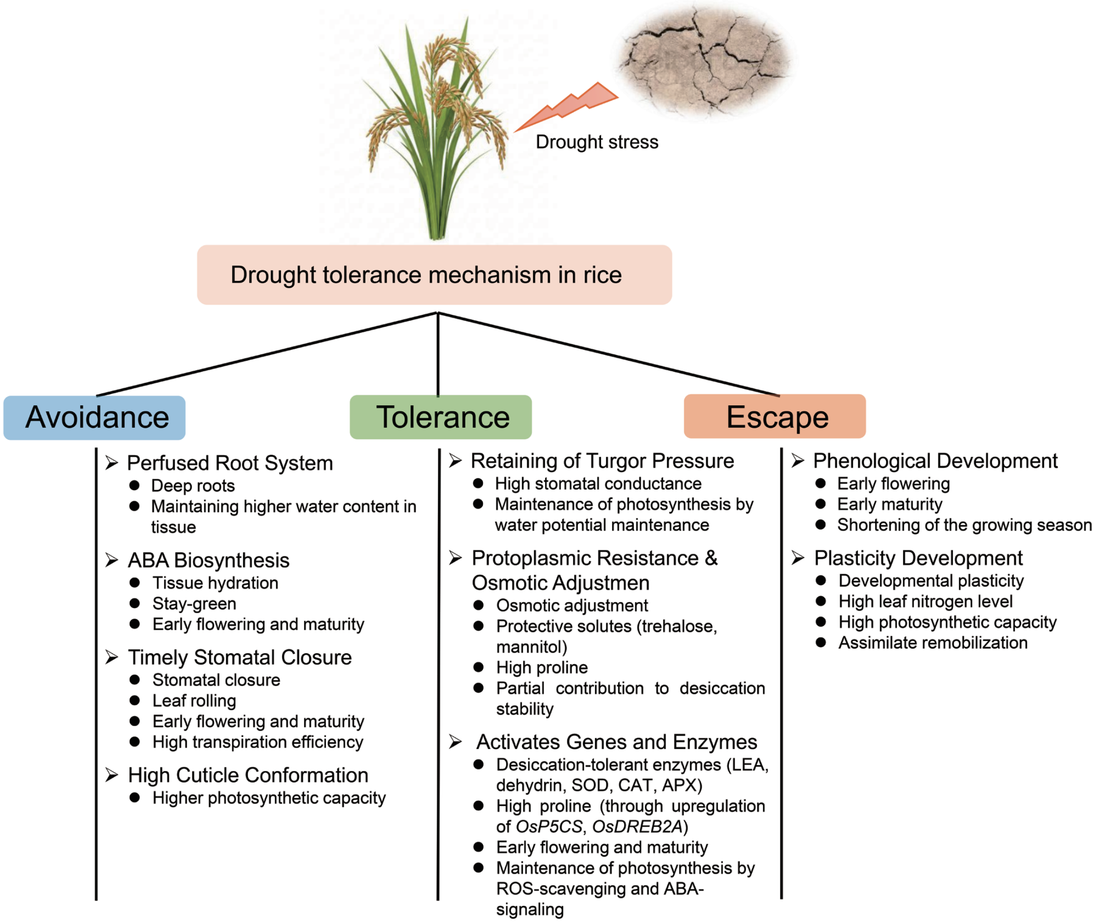

圖 1. 植物於乾旱逆境的反應。

Fig. 1. The responses of plants under drought stress. LEA: late embryogenesis abundant; SOD: Superoxide dismutase; CAT: catalase; APX: ascorbate peroxidase; *OsP5CS*: *Oryza sativa* delta-1-pyrroline-5-carboxylate synthase; *OsDREB2A*: *Oryza sativa* drought-responsive element binding protein 2A.

應機制，作為後續耐旱特性研究與相關分子育種策略的重要理論基礎。

水稻乾旱逆境下之型態與解剖構造變化

水稻葉片在乾旱逆境下的形態與解剖學的反應機制

乾旱逆境水稻植株因土壤水勢 (water potential) 的下降，導致木質部水分的傳輸受阻，致使葉細胞無法維持正常的膨壓 (turgor pressure)，進而抑制其生長與細胞擴張，導致劍葉顯著縮小 (Hussain *et al*. 2018)。水分供應的不足，不僅影響劍葉的正常發育，也改變其組織結構與超微形態，包括：葉面積縮小、氣孔密度減少、細胞壁增厚、角質層沉積增加，及維管傳導組織的發育異常 (Upadhyaya & Panda 2019)。在外觀表型上，LR 與劍葉早期衰老 (leaf senescence)，常被視為水稻對乾旱逆境的典型反應，亦是田間篩選耐旱品種 (系) 的重要外觀參考指標。此外，葉片諸多相關性狀，已被證實可反應水稻對乾旱的耐受能力，包括：劍葉面積 (flag leaf area)、葉面積指數 (leaf area index; LAI)、葉片相對含水量 (relative water content; RWC) 及葉片色素含量 (leaf pigment content) 等 (表 1)。這些性狀在

表 1.　葉片表型性狀指標與水稻耐旱特性的對照。

Table 1.　Leaf phenotypic trait indices in relation to the drought tolerances of rice[z].

Indicator name	Definition	Physiological significance	Application in drought tolerance selection
Flag leaf area	Leaf area of the main panicle flag leaf (cm^2)	Determines photosynthetic potential and nutrient supply to the panicle	Larger flag leaf area enhances photosynthesis and grain filling under drought conditions
LAI	Total leaf area per unit ground area (m^2/m^2)	Reflects canopy light interception efficiency and transpiration capacity	Moderate LAI helps balance water use; overly high LAI may cause excessive water loss
RWC	(Fresh weight − Dry weight)/(Turgid weight − Dry weight) × 100%	Evaluates water retention ability of leaf tissues	High RWC indicates good water status and serves as an important drought tolerance indicator
LR	Longitudinal curling of leaves caused by decreased turgor pressure	Morphological adaptation to drought, reducing transpiration	Widely used for rapid field drought scoring (scale 1–9)
Leaf thickness and cell wall thickening	Leaf cross-sectional thickness and cell wall structural changes	Influences water retention and mechanical support	Thicker leaves delay water loss but must balance photosynthetic efficiency
Leaf pigment content (Chlorophyll)	Concentration of chlorophyll a/b and total pigments	Indicates photosynthetic capacity and leaf health status	Chlorophyll reduction is an early drought stress signal, useful for non-destructive monitoring
Early leaf senescence index	Rate of leaf chlorosis, yellowing, and necrosis	Reflects nutrient remobilization under drought	Indicates grain filling potential, positively correlated with drought tolerance
Stomatal density	Number of stomata per unit leaf area	Regulates gas exchange and water transpiration	Lower density reduces water loss, acting as a drought adaptation strategy
Cuticle development	Thickness of waxy and cuticular layers on leaf surface	Enhances resistance to water loss and mechanical protection	Closely related to drought avoidance strategies
Stomatal conductance	CO^2 and water vapor permeability influenced by stomatal aperture regulation	Balances photosynthesis and water loss	Key physiological parameter measured with precision instruments, can be integrated with genotype analysis

[z] LAI: leaf area index; RWC: relative water content; LR: leaf rolling; CO^2: carbon dioxide.

乾旱條件下可呈現顯著變異，並已建立評分分級系統，廣泛應用於水稻耐旱品種 (系) 之田間選拔與分子育種選拔策略 (Hirooka *et al*. 2017; Swapna & Shylaraj 2017; Kumar *et al*. 2021; Gunnula *et al*. 2022)。

水稻乾旱逆境下的根系形態與生理特性

作物根系的形態與功能，是乾旱逆境作物維持水分吸收與產量穩定的關鍵因子。而水稻面臨水分缺乏之時，其產量的表現與根系構造特性及生長動態，有密切的關聯性。近來研究指出，根系多樣性 (如乾重或總長度) 可作為評估乾旱逆境水稻之生產潛力的預測指標 (Kim *et al*. 2020)，爰此已有學者進一步觀察不同水稻品種 (系) 在乾旱下的根系表型外觀反應，藉此評估其耐旱潛力與適應策略。另一方面，有學者發現，水稻在乾旱逆境下，根系會因 ABA 濃度的上升，誘導其根系加長，以提高深層土壤水分的吸收能力。整體而言，具備較深厚根系、粗壯主根、分支能力強及高根莖比 (root/shoot ratio) 等根系性狀的水稻品種 (系)，通常具有較佳的乾旱適應性與穩定產量 (Mishra *et al*. 2019; Kim *et al*. 2020; Ramachandran *et al*. 2021)。因此，水稻根系的形態與生理性狀，在乾旱逆境下，不僅影響水稻苗期的初始生長，亦與後期穀物產量的形成有密切關聯性，故是耐旱品種選拔的重要依據之一。

目前水稻根系的表型分析，多將根系區分為上部 (根冠至近地表層)、中部 (主根與次生根中段) 及下部 (主根延伸區與部分深層側根) 3 個根區，經取樣清洗乾淨後，再透過影像分析工具進行根性系統結構的量測。常見根系掃描與分析軟體，包括：WinRhizo、RhizoVision Explorer 及 ARIA (Automatic Root Image Analysis) 等 (Li *et al*. 2022)。這些工具可量化根長 (root length)、根表面積及根容積等重要參數，提供高通量且客觀地根系性狀量測的結果，有助於加速耐旱相關數量性狀基因座 (qualitative trait loci; QTLs) 探勘與輔助耐旱育種篩選等應用。

水稻乾旱逆境下之生理反應與細胞膜功能調控

乾旱逆境對水稻光合作用之效應

乾旱逆境會顯著削弱水稻的光合作用速率與氣體交換能力。當植株缺水時，葉片氣孔雖能藉閉合減少蒸散作用，但同時限制 CO_2 進入葉肉細胞，使 CO_2 同化速率下降。此限制造成電子傳遞鏈中電子累積，促使活性氧族 (reactive oxygen species; ROS) 生成，進一步引發光合組織氧化逆境 (Zhu *et al*. 2020)，並損害光合作用結構與功能，包括：光捕獲效率降低、Rubisco 之酵素活性減弱，以及葉綠素含量減少 (Panda *et al*. 2021)。對缺水敏感的水稻品種 (系) 而言，光合作用受限，加上根區水分利用率的下降，會造成顯著產量損失 (Gupta *et al*. 2020; Zhu *et al*. 2020)。在光系統層級，乾旱逆境會造成光系統 I (photosystem I; PSI) 與光系統 II (photosystem II; PSII) 運作的破壞，尤其 PSII 對電子傳遞與腺苷三磷酸 (adenosine triphosphate; ATP) 合成扮演著至關重要的角色。乾旱逆境會降低水稻光反應與氧氣釋放的效率，致使電子傳輸鏈受抑制並導致 PSII 失去活化 (Mishra *et al*. 2019)。

葉綠素作為光合作用的核心色素，負責捕獲光能並轉化為還原力。然而缺水會降低葉肉細胞對 CO_2 的利用潛力，減少活性葉綠素含量，並導致最大 PSII 量子產率 (Fv/Fm) 的下降 (Zhu *et al*. 2020; Guo *et al*. 2022; Arief *et al*. 2023)。此外，類胡蘿蔔素在光保護中亦扮演關鍵角色，不僅能吸收過量光能避免光抑制外，亦為多種逆境反應訊號分子的前驅物 (Swapnil *et al*. 2021)。因此，利用分子育種或基因工程提升作物的類胡蘿蔔素含量，已成為目前強化作物乾旱適應性或光保護能力的潛在策略 (Gao *et al*. 2023; Kumar & Sindhu 2024; Nazari *et al*. 2024)。

乾旱逆境對水稻細胞膜功能之效應

水稻與環境水分間的關係，常以水勢能 (water potential) 與 RWC 作為核心指標予以描

述 (Gupta *et al*. 2020)。其中，水分利用效率 (water use efficiency; WUE) 被視為水稻在水分逆境下決定產量潛力的關鍵組成因子，亦是提升水稻耐旱能力的重要策略之一 (Mishra *et al*. 2019)。RWC 則為評估水稻植株體內水分的最佳綜合性測量值，能反應植株體內水勢能與細胞膨壓的變化情形。當水稻暴露於乾旱逆境時，常呈現 RWC 下降、膨壓降低及蒸散作用受抑等負面生理效應 (Gupta *et al*. 2020)。在蛋白質體研究方面，有學者以 4 週齡耐旱水稻幼苗為材料進行篩選，發現供試材料在缺水處理 48–72 時期間，因脯氨酸含量上升而促進滲透調節，使 RWC 呈現穩定上升趨勢 (Medha *et al*. 2021)。脯氨酸在滲透調節中扮演關鍵角色，不僅有助於維持葉片膨壓，亦能減緩水分逆境對細胞功能的損害。

另一方面，細胞膜穩定性 (cell membrane stability; CMS) 於乾旱逆境下的維持，已被廣泛認為是植物耐旱性的重要生理指標。乾旱逆境會使膜系統的水分被排除，導致脂質結構受損與錯位，進而增加細胞膜的通透性，使植體在脫水過程中更易喪失細胞完整性 (Singh *et al*. 1992; ElBasyoni *et al*. 2017)。細胞膜穩定性指數 (membrane stability index; MSI) 可依據 Blum & Ebercon (1981) 的方法進行測定，而該指數已被應用於探討水稻在乾旱逆境下的膜穩定性與產量表現之間的相關性 (Blum & Ebercon 1981; Upadhyaya & Panda 2019)。

水稻乾旱逆境下之生化調節與抗氧化防禦系統

在乾旱逆境下，水稻可透過有機與無機滲透性溶質 (osmolytes) 的累積，減緩細胞內滲透壓，以維持細胞膨壓與基本生理功能。目前所發現的滲透保護劑包括：脯氨酸、甘氨酸甜菜鹼 (glycine betaine) 及各類可溶性糖。這些物質可透過滲透調節 (osmotic adjustment)，有效減緩脫水所造成的細胞損傷 (Kumar *et al*. 2017; Upadhyaya & Panda 2019)。另，細胞內蛋白質含量與組成的變化，配合抗氧化防禦系統的的活性提升，亦有助於強化水稻耐旱特性 (Pandey & Shukla 2015)。此外，學者亦發現有些植物激素，如 ABA、油菜素內酯 (brassinosteroids; BRs) 及乙烯 (ethylene)，於特定組織與時序的專一性表達，通常亦能有效促進植株的乾旱適應性，而不犧牲最終產量 (Gupta *et al*. 2020; Haghpanah *et al*. 2024)。

<u>乾旱逆境下滲透物質的累積</u>

滲透調節為植物耐旱的核心生理過程之一 (圖 1)。當葉細胞膨壓下降時，植株會誘導滲透保護劑累積，以維持水分平衡與細胞完整性。以玉米為例，缺水處理可誘導脯氨酸、可溶性糖、酚類化合物及總游離胺基酸等的累積，並顯著提升耐旱能力 (Anjum *et al*. 2017)。脯氨酸是一種五碳胺基酸，兼具滲透調節與保護細胞結構功能，亦可作為篩選耐旱品種 (系) 的生化標記 (Hayat *et al*. 2012; Pandey & Shukla 2015; Mishra *et al*. 2019; Medha *et al*. 2021)。糖類除作為能量與結構來源外，亦參與光合作用與呼吸作用的調控。在乾旱逆境中，水稻會累積雙醣、低聚醣、果聚醣等可溶性糖及特定糖醇，如甘露醇、山梨糖醇及海藻糖，並被證實能增強水稻的耐旱性 (Benkeblia 2022)。尤其，學者利用代謝體學研究分析發現，水稻於乾旱逆境下，會累積如脯胺酸 (proline)、海藻糖 (trehalose) 及棉子糖 (raffinose) 等滲透保護劑，同時伴隨 ABA 與茉莉酸 (jasmonic acid) 等信號分子的變化 (Alcázar *et al*. 2010; Kajrolkar 2025)。綜合而言，脯氨酸與可溶性糖的協同累積，是水稻在乾旱逆境維持生理活性的重要策略 (Upadhyaya & Panda 2019)。進一步整合蛋白質體學 (proteomics) 與代謝體學研究研究結果，學者發現乾旱逆境下的玉米，會調整其代謝過程，傾向於胺基酸與有機酸的分解代謝，以滿足在水分虧缺下的能量需求 (Witt *et al*. 2012)。而這些針對乾旱逆境下植物代謝重構的見解，可對未來進行靶向性代謝工程提高作物缺水耐受性，提供重要的關鍵基礎。

<u>乾旱逆境下 ROS 的產生</u>

ROS 為植物有氧代謝的天然副產物，但於乾旱逆境下，其生成速率顯著增加，將對細胞造成氧化損傷。ROS 主要在光合作用與呼吸作用過程中，分別於葉綠體與粒線體中

所產生 (Gill & Tuteja 2010)。然而，乾旱逆境會抑制植物細胞的電子傳遞鏈，導致超氧自由基 (superoxide; $O_2{\bullet}^-$)、過氧化氫 (hydrogen peroxide; H_2O_2) 及羥基自由基 (hydroxyl radical; OH•) 的大量累積 (Melandri *et al*. 2020; Fathi *et al*. 2025)。過量 ROS 進而會氧化蛋白質、脂質及 DNA，造成光合作用與光呼吸的破壞，並引發脂質過氧化與細胞膜結構的崩解，最終導致細胞死亡 (圖 2) (Melandri *et al*. 2020; Fathi *et al*. 2025)。因此，降低 ROS 產生與增強抗氧化系統則是另一提升水稻耐旱性的關鍵策略。

乾旱逆境下抗氧化物質的角色

植物具備複雜的抗氧化防禦系統，可分為酶類抗氧化物與非酶類抗氧化物 2 大類 (Gill & Tuteja 2010；圖 2)。其中，酶類抗氧化物包括：超氧化物歧化酶 (superoxide dismutase; SOD)、過氧化氫酶 (catalase; CAT)、穀胱甘肽過氧化物酶 (glutathione peroxidase; GPX)、抗壞血酸過氧化物酶 (ascorbate peroxidase; APX)、單脫氫抗壞血酸還原酶 (monodehydroascorbate reductase; MDHAR)、脫氫抗壞血酸還原酵素 (dehydroascorbate reductase; DHAR) 及穀胱甘肽還原酶 (glutathione reductase; GR)；非酶類

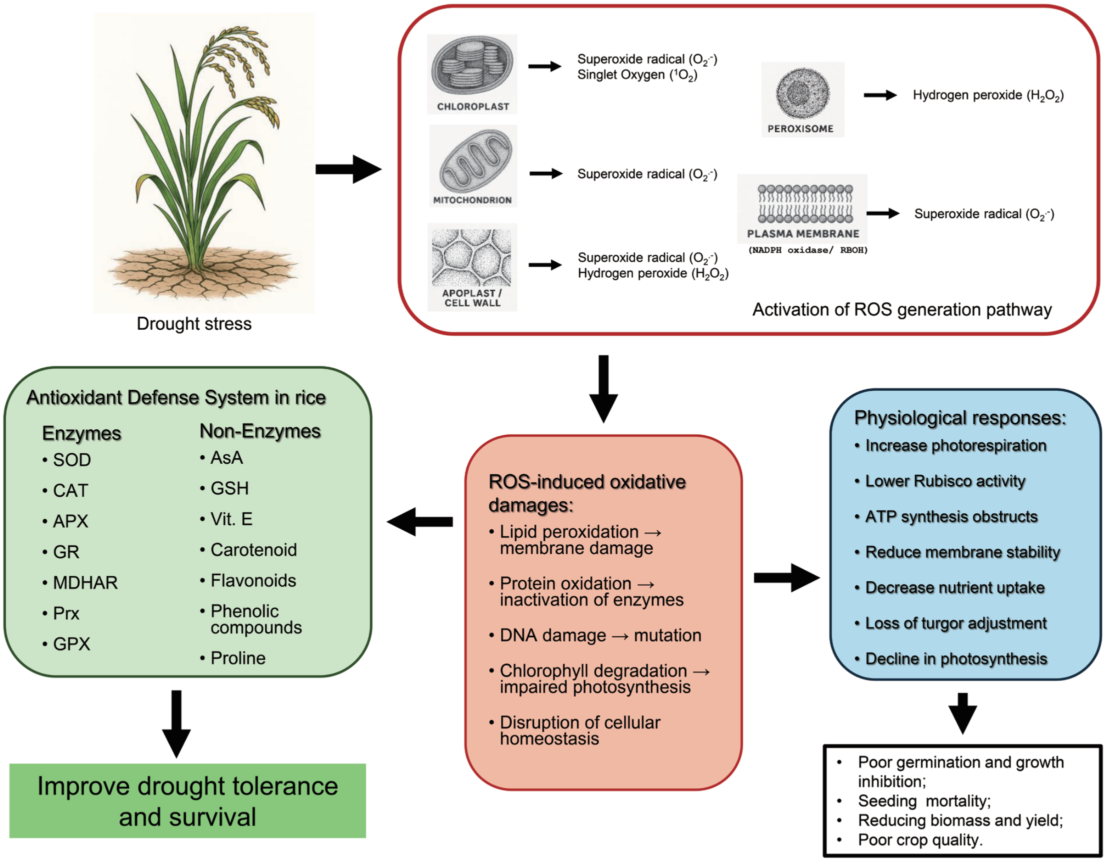

圖 2. 水稻乾旱逆境之活性氧族所造成的損害及其抗氧化防護機制。

Fig. 2. Reactive oxygen species (ROS)- induced cellular damage and antioxidant defense mechanisms in rice under drought stress. SOD: superoxide dismutase; CAT: catalase; APX: ascorbate peroxidase; GR: glutathione reductase; MDHAR: monodehydroascorbate reductase; Prx: peroxidases; GPX: guaiacol peroxidase; AsA: ascorbic acid; GSH: glutathione; Vit. E: vitamin E; ATP: adenosine triphosphate.

抗氧化物包括：抗壞血酸 (ascorbic acid; AsA) 與穀胱甘肽 (glutathione; GSH)。SOD 是 ROS 清除的第一道防線 (Gill & Tuteja 2010)，在乾旱逆境處理的水稻與小麥植株，常誘導其活性的上升 (Csiszar *et al*. 2005; Zlatev *et al*. 2006; Lü *et al*. 2010; Mishra & Panda 2017; Melandri *et al*. 2020)。CAT 負責分解過氧化氫，水稻於乾旱逆境下可調整其活性，但活性下降是水稻對多種逆境的常見反應 (Qureshi *et al*. 2018)；GPX 則可有效清除氧化氫，其活性已被當作水稻耐旱的重要指標 (Bhattacharje & Dey 2018; Qureshi *et al*. 2018; Bhandari *et al*. 2023)。

位於葉綠體的 AsA–GSH 循環 (圖 2) 是過氧化氫清除的核心路徑，關鍵酶如 APX 與 GR 在乾旱下活性增強，有助於維持細胞氧化還原平衡並增強植物抗旱特性 (Bhattacharjee & Dey 2018; Upadhyaya & Panda 2019; Fathi *et al*. 2025)。增加 AsA 與 GSH 含量與耐旱性間具有高度的關聯性，其已被證實可有效降低乾旱所造成的產量損失 (Bhattacharjee & Dey 2018; Melandri *et al*. 2020)。因此，強化抗氧化代謝相關酶類與非酶類成分的表現，已成為耐旱水稻分子育種的重要策略之一。

植物耐旱分子調控機制

植物對乾旱的反應涉及多維度的複雜訊號傳遞：自細胞膜受器感知乾旱逆境後，透過訊息傳導，啟動耐旱相關基因的表現 (圖 3；Dash *et al*. 2018; Oladosu *et al*. 2019; Sonowal *et al*. 2025)。由於耐旱性狀複雜，致傳統雜交與選拔程序，常難以精確評估育種族群的耐旱特性。但分子標記技術則可提高篩選效率，或可應用於耐旱種原或品種的選拔。因此，研究者須致力於建立與耐旱相關的 QTLs (Barik *et al*. 2019; Upadhyaya & Panda 2019)，並配合發展田間自動化高通量的耐旱外表型分析方法 (Prakash *et al*. 2019)。

水稻耐旱 QTLs 定位研究現況與限制

QTLs 指在基因組上控制連續變異 (數量性狀) 的基因座。過去分子遺傳研究已在不同遺傳族群、生長階段與環境條件下，探勘出多個與乾旱相關的典型 QTLs (見表 2)，例如與產量、收穫指數、葉片含水量、捲葉度及稔實率相關者 (Alexandrov *et al*. 2015; Todaka *et al*. 2015; Dixit *et al*. 2017; Barik *et al*. 2019)。其中，連結產量表現的耐旱 QTLs (如 qDTYs、$qDTR_8$ 及 $qDLR_{8.1}$ 等)，在實務育種上備受重視，常與簡單序列重複 (simple sequence repeat; SSR) 等共顯性標記緊密連鎖，可用於早期世代的耐旱基因型篩選 (Mishra *et al*. 2013; Dixit *et al*. 2014; Ramchander *et al*. 2016; Singh *et al*. 2016; Dixit *et al*. 2017; Vinod *et al*. 2019; Venkateshwarlu *et al*. 2025)。此外，Barik *et al*. (2019) 更發現 qLR9.1 等 QTLs 可控制乾旱下的卷葉、乾重、收穫指數、穗花稔實率及含水量等性狀，顯示其在耐旱育種上具有高度應用潛力 (Barik *et al*. 2019)。

但目前多數已發表 QTLs 遺傳區間的距離仍較大，或有遺傳累贅的問題，或因其性狀解釋力深受環境影響，且其上關鍵基因尚未被選殖，均嚴重影響這些 QTLs 在耐旱育種選拔程序上的應用性 (Singh *et al*. 2016)。因此，未來或可進一步將相關 QTLs 進行細定位，或結合高通量自動化表型體 (automative phenomics) 分析技術與多環境田間試驗驗證，應是水稻耐旱特性研究由實驗室證據走向育種落地的關鍵。

現已選殖水稻耐旱基因

乾旱可誘導水稻發生大規模轉錄變化：約有 5,000 個基因上調表現，6,000 個下調表現 (Bin Rahman & Zhang 2016; Joshi *et al*. 2016)。現已知耐旱基因可分為 3 類 (見表 3 與圖 3)，彼此交互作用而共同塑造乾旱下的水稻生理/生化反應：(1) 膜轉運相關：調節離子/溶質運輸與滲透調節，支撐葉片含水與細胞膨壓維持；(2) 訊號轉導相關：包含 *CDPK* (calcium-dependent protein kinase) 與 *CIPK* (CBL-interacting protein kinase) 等，整合鈣訊號、ABA 及 ROS 的穩定狀態，快速驅動下游反應 (如 *CDPK7* 與 *CIPK03/12* 等) (Sahebi *et al*. 2018; Bandurska 2022)；(3) 轉錄調控相關：如 *DREB*/*CBF*、*NAC* 及 *WRKY* 等家族，

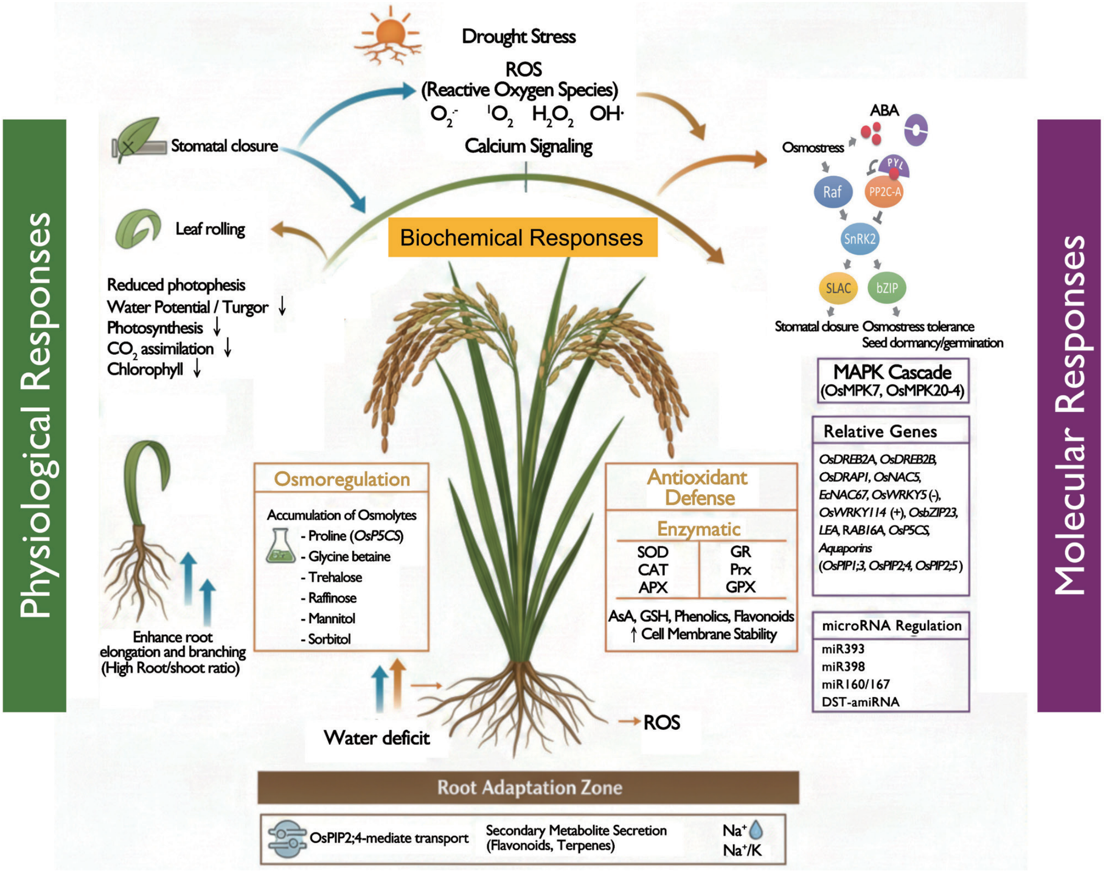

圖 3. 水稻在乾旱逆境下生理、生化與分子層次反應。

Fig. 3. The physiological, biochemical, and molecular responses of plants to drought stress. SOD: superoxide dismutase; CAT: catalase; APX: ascorbate peroxidase; GR: glutathione reductase; MDHAR: monodehydroascorbate reductase; Prx: peroxidases; GPX: guaiacol peroxidase; AsA: ascorbic acid; GSH: glutathione; OsMAPK7: *Oryza sativa* mitogen-activate protein kinase 7; OsMAPK20-4: *Oryza sativa* mitogen-activate protein kinase 20-4; *OsDREB2A*: *Oryza sativa* drought-responsive element binding protein 2A; *OsDREB2B*: *Oryza sativa* drought-responsive element binding protein 2B; *OsDRAP1*: *Oryza sativa* drought responsive AP2/EREBP 1; *OsNAC5*: *Oryza sativa* NAC-type transcription factor 5; *EcNAC67*: *Eleusine coracana* NAC transcription factor 67; *OsWRKY5*: *Oryza sativa* WRKY transcription factor 5; *OsWRKY14*: *Oryza sativa* WRKY transcription factor 14; *OsbZIP23*: *Oryza sativa* basic leucine zipper 23; *LEA*: late embryogenesis abundant gene; *RAB16A*: responsive to ABA gene 16A; *OsP5CS*: *Oryza sativa* delta-1-pyrroline-5-carboxylate synthase; *OsPIP1;3*: *Oryza sativa* plasma membrane intrinsic protein 1;3; *OsPIP2;4*: *Oryza sativa* plasma membrane intrinsic protein 2;4; *OsPIP2;5*: *Oryza sativa* plasma membrane intrinsic protein 2;5.

直接或間接調控抗逆效應基因之表達 (Dash *et al*. 2018; Upadhyaya & Panda 2019; Gupta *et al*. 2020; Kim *et al*. 2020)。

目前已被驗證的水稻耐旱功能性基因包括：*LEA*、*OsDREB2B* 及 *OsDRAP1* 等基因，可促進滲透調節保護與根系適應 (Dash *et al*. 2018)；在對伊拉克水稻品種的乾旱耐受性評估中，耐旱性最強的品種 'Mashkab' 顯示具有較高的 *OsDREB2A* 表現，暗示 *OsDREB2A* 表達與水稻耐旱性之間存在潛在關聯 (Al Azzawi *et al*. 2020; Gammanpila *et al*. 2025)。另一方面，*OsDREB2A* 的大量表現，已在轉譯層面證實能增強水稻的耐旱性 (Cui *et al*. 2011; Gammanpila *et al*. 2025)；*OsPYL/RCAR5* 與 *EcNAC67* 可提升根與芽之生長動態 (Rahman *et al*. 2016; Kim *et al*. 2020)；*OsNAC5* 則可促進水稻提高 WUE、

表 2. 水稻已發表之與耐旱相關的數量性狀基因座。

Table 2. The published drought-related quantitative trait loci (QTLs) in rice.

QTL	Chromosome	Marker interval	Parents	Associated traits	Reference
$qDTY_{1.1}$	1	RM431-RM104	Dhagaddeshi × Swarna	Grain yield and stem dry weight under drought	Venkateshwarlu *et al*. (2025)
$qDTY_{2.1}$	2	RM154-RM324	Aus × IR64	Grain yield and days to heading under drought	Sandhu *et al*. (2014)
$qDTY_{2.2}$	2	RM211-RM263	Kali Aus/2 × MTU1010	Grain yield under drought	Sandhu *et al*. (2014)
$qDTY_{2.3}$	2	RM263-RM573	Kali Aus/2 × MTU1010	Grain yield under drought	Palanog *et al*. (2014)
$qDTY_{3.1}$	3	RM168-RM468	TDK1 × IR55419-04	Grain yield under drought	Dixit *et al*. (2014)
$qDTY_{3.2}$	3	RG191-RM546	Danteshwari × Dagaddeshi	Grain yield under drought	Panda *et al*. (2021)
$qDTY_{6.1}$	6	RM168-RM468	TDK1 × IR55419-04	Grain yield under drought	Dixit *et al*. (2014)
$qDTY_{8.1}$	8	RM339-RM210	Swarna × Basmati 334	Grain yield and grain number under drought	Venkateshwarlu *et al*. (2025)
$qDLR_{8.1}$	8		IRAT109 × Yuefu	Leaf rolling	Qu *et al*. (2008)
$qDTR_{8}$	8	RM264-RM20a	Norungan × TKM9 BC_1F_5	Drought response index (composite)	Ramchander *et al*. (2016)
$qLR_{9.1}$	9	RM316-RM257	CR 143-2-2 × Krishnahamsa F_7	Leaf rolling, leaf drying, relative water content, spikelet fertility, harvest index	Barik *et al*. (2019)
$qDTY_{12.1}$	12	RM28166-RM28199	IR74371-46-1-1 × Sabitri	Grain yield under drought	Mishra *et al*. (2013)

表 3. 水稻基因組中已選殖之耐旱功能性基因。

Table 3. The cloned functional genes related with drought-response in rice genome.

Gene name	Gene source	Promoter	Transgenic construct[z]	Associated drought-related traits	Accession number	Reference
OsLEA3-1	Rice	HVA1/ CaMV 35S/ actin	OE	Increased yield	LOC_Os05g46480	Xiao *et al*. (2007)
CIPK03/CIPK12	Rice	Ubiquitin	OE/RNAi	Regulatory protein, signal transduction, protease; RNAi lines showed reduced fertility	AK072868 (AP003757); AK101442 (AP003409)	Xiang *et al*. (2007)
CDPK7	Rice	CaMV 35S	OE	Regulatory protein, signal transduction, protease	AB042550	Saijo *et al*. (2000)
OsITPK2/DSM3	Rice	CaMV 35S/ Ubiquitin	OE/amiR	Low fertility, reduced biomass	LOC_Os03g12840	Du *et al*. (2011)
OsDREB2A	Rice	4ABRC	OE	Enhanced survival rate of transgenic plants	Os01g0165000	Zhang *et al*. (2013)
OsNAC5	Rice	RCc3/ GOS2	OE	Increased root diameter, improved yield	AK102475	Jeong *et al*. (2013)
OsCPK9	Rice	CaMV 35S	OE	Enhanced stomatal closure for improved drought resistance and better osmotic adjustment in transgenics	LOC_Os03g48270	Wei *et al*. (2014)
OsPYL/RCAR5	Rice	GUS	OE	Inhibits growth	Os05g12260	Kim *et al*. (2014)
EcNAC67	Millet	RD29	OE	Increased relative water content, delayed leaf rolling, higher root and shoot biomass	KU500625	Rahman *et al*. (2016)
MAPK gene family	Rice	Ubi	OE	Regulatory protein, signal transduction		Huang *et al*. (2021a)
WRKY gene family	Rice	CaMBP/ CRSSP	OE/OX	Relative yield reduction		Raineri *et al*. (2015); Huang *et al*. (2021a); Lim *et al*. (2022); Song *et al*. (2022); Li *et al*. (2025)
OsPIP gene family	Rice		OE	Regulatory protein, signal transduction, and better osmotic adjustment		Verma *et al*. (2020); Bai *et al*. (2021); Huang *et al*. (2021b); Sun *et al*. (2021); Tong *et al*. (2025); Tran *et al*. (2025)

[z] OE: over expression; RNAi: RNA interference; amiR: artificial miRNA; OX: knock out.

光合作用及抗氧化酵素活性 (Khan *et al*. 2025)；水稻 *NAC* 轉錄因子家族透過調控與光合作用效率及碳同化相關的基因，在水稻面對多樣環境挑戰時扮演了優化碳固定的角色 (Gammanpila *et al*. 2025)；*OsCPK9* 可促進水稻強化氣孔關閉動力學；*OsDREB2A* 增強水稻乾旱與鹽逆境下的存活率 (Liu *et al*. 2014)；*OsP5CS* 表達增加有助於植物產生更多的脯氨酸，從而使植物免受乾旱影響 (Saddique *et al*. 2020)；*WRKY* 與 *OsITPK2* 亦參與水稻耐旱反應訊號整合與 ROS 平衡 (Du *et al*. 2011; Sahebi *et al*. 2018)；此外，水稻 *WRKY* 轉錄因子在調控水稻耐旱性方面展現出多重且複雜的機制，特別是其對 ABA 訊號、ROS 穩態及氣孔性能的影響 (Li *et al*. 2025)；*OsWRKY5* 負向調控 *OsMYB2* 的表現，進而抑制多個乾旱反應基因，包括 *OsLEA3*、Os*RAB16A* 及 *OsDREB2AI* 表現，而負調控水稻的耐旱反應 (Lim *et al*. 2022)。另一方面，*MAPK* 蛋白基因 (*OsMPK7*、*OsMPK9*、*OsMPK20-1* 及 *OsMPK20-4*) 可活化 *OsWRKY55* 的表現，進而加速植物體的水分流失與活化氧族累積 (Huang *et al*. 2021a)。此外，大量表現 *OsWRKY114* 亦會抑制 ABA 受體基因—*OsPYL2* 與 *OsPYL10* 的表現，進而限制氣孔的關閉 (Song *et al*. 2022)。

水稻中的水通道蛋白 (aquaporins; AQPs) 是水稻調控水分運輸的關鍵膜蛋白，對乾旱、鹽分及高溫逆境下的植株適應性，具有非常重要的角色 (Tong *et al*. 2025)。尤其，特定 AQPs 亞家族成員在耐旱機制中，是非常重要的，例如 *OsPIP2* 亞家族成員 (如 *OsPIP2;2*、*OsPIP2;3* 及根特異性表達的 *OsPIP2;4*) 能顯著提高水分吸收與運輸效率 (Bai *et al*. 2021; Sun *et al*. 2021; Tran *et al*. 2025)。其中，*OsPIP2;4* 甚至兼具運輸水分與非選擇性 Na^+、K^+ 離子的功能，有助於滲透調節 (Tran *et al*. 2025)。此外，在耐旱品種中，多個 AQPs 基因，包括：*OsPIP1;3*、*OsPIP2;4*、*OsPIP2;5*、*OsTIP2;1* 及 *OsNIP2;1*，在乾旱時會更強烈地協同增加表現量，顯示水稻的耐旱性是一種由多基因決定的複雜性狀 (Verma *et al*. 2020)。進一步利用 CRISPR/Cas9 準基因編輯技術，針對 *OsPIP1* 基因族進行多重編輯，證實了這些 AQPs 基因在維持滲透穩態中的關鍵作用 (Huang *et al*. 2021b)。目前這些基因大多於溫室或控環環境中被證實具耐旱效益，但要達到育種可信的增益，仍需在多點多年田間試驗結合表型體與環境共變量進行驗證，並評估其對產量構成與資源分配的長期影響。

microRNA 在水稻乾旱耐性的角色

miRNA 是一類小型非編碼 RNA，透過轉錄後抑制調控系統性的乾旱反應相關基因，水稻中已報導多條 miRNA 與乾旱功能相關 (Singh *et al*. 2020)。研究顯示，miR393、miR319 及 miR397 在乾旱下上調表現 (Sunkar & Zhu 2004)；miR393 透過調控 *OsAUX1* 與 *OsTIR1* 影響分櫱、開花期及生長素反應 (Xia *et al*. 2012)。水稻中另已發現逾 30 種與乾旱相關的 miRNA，19 種參與乾旱調控 (Zhou *et al*. 2010)；在 ‘Nagina 22’ (N22) 中更鑑定 71 種新型 miRNA，顯示遺傳背景對 miRNA 網絡的調控表現作用顯著 (Mutum *et al*. 2016)。特定 miRNA，如 miR398 調控 *Cu*/*ZnSOD*，影響抗氧化能力 (Balyan *et al*. 2017; Lu *et al*. 2022)；miR160/miR167 作用於 *ARF*，參與早期生長素訊號與根系建構 (Upadhyaya & Panda 2019)。其他如 DST-amiRNA 與 ROS 穩定狀態相關基因，能促進氣孔關閉與密度下降，增強耐旱性 (Faisal *et al*. 2017)。miR169-3p 與 miR166e-3p 則與根系發育及碳水化合物代謝有關。這些 miRNA 為解析水稻耐旱機制與發展分子育種提供了關鍵工具與潛力標的 (Cheah *et al*. 2015; Upadhyaya & Panda 2019; Singh *et al*. 2020)。

多體學研究在乾旱逆境研究中的最新進展

多體學 (multi-omics) 研究策略是整合不同維度分子體學數據的系統生物學方法，可用來全面剖析植物於乾旱逆境下的分子與生理機制，進而為未來氣候適應型作物 (climate-resilient crops) 的育種工作，提供精準研究與應用路徑的基礎。其核心理念是透過基因體、轉錄體、蛋白體、代謝體、脂質體、離子體及表觀體等，各種體學資料的整合分析，進而描繪出植物乾旱反應的系統性網絡 (system-level drought response network；圖 3)，進而連結基

因調控、代謝重構及表型表現之間的層級關係 (Gupta *et al*. 2023; Goche *et al*. 2025; Kajrolkar 2025; Sonowal *et al*. 2025)。

透過高通量基因體與轉錄體的整合分析，可明確勾勒出植物耐旱性狀的多基因特徵與調控邏輯。尤其，全基因組關聯研究 (genome-wide association study; GWAS) 與功能基因體學的進展，使得研究者能深入研究植物耐旱關鍵突變位點與基因調控網絡。學者結合長片段測序 (long-read sequencing; LRS) 與單倍型解析 (haplotype phasing) 分析結果，可精準定位耐旱 QTL 區段，並預測候選基因的表達模式 (Yadav *et al*. 2019; Shinozaki & Yamaguchi-Shinozaki 2022)。此外，可利用基因編輯技術 (如 CRISPR/Cas9 與 prime editing)，針對耐旱相關調控因子，如 *DREB*、*NAC* 及 *bZIP* 家族基因，進行功能驗證，證實其在耐旱性狀扮演重要角色外，亦初步實現作物耐旱性狀的定向改造 (targeted improvement; Kajrolkar 2025)。

在水稻轉錄體與代謝體整合分析結果發現，乾旱逆境可誘導水稻 *OsDREB1C*、*OsNAC6* 及 *OsPP2C*，並重新平衡碳水化合物代謝與 ROS 代謝，強化細胞之能量穩態與抗氧化防禦 (Goche *et al*. 2025)。同時，在玉米與小麥乾旱逆境的轉錄體及代謝體的分析結果，亦發現能量轉換與滲透壓調節代謝路徑的重塑，應是植物的主要乾旱逆境反應模式 (Hashiguchi & Komatsu 2016)。此外，蛋白質體學通過質譜技術，呈現蛋白質豐度與轉譯後修飾 (post-translational modifications)，如磷酸化與蛋白質–蛋白質交互作用 (protein-protein interactions) 的動態變化，並發現 MAPK (mitogen-activated protein kinases) 訊息路徑、離層素相關調控 (ABA-dependent regulation) 及脂質重構 (lipid remodeling)，在植物乾旱逆境反應中，互為支撐 (Todaka *et al*. 2015; Kim *et al*. 2022; Li *et al*. 2023)。此類多維度跨體學資料的整合，使研究者得以建立分子訊號 – 代謝反應 – 表型表現的連續鏈結，為後續抗旱育種提供可量化之生物標誌 (biomarkers)。

同時，多體學亦揭露植物藉由多層次調控，進行代謝重構 (metabolic reprogramming)，以維持逆境下的內穩態 (homeostasis) (Lisec *et al*. 2006)。在水稻代謝體分析，顯示胺基酸 (如脯胺酸及丙胺酸 (alanine)) 及糖醇類 (如山梨醇 (sorbitol)、甘露醇 (mannitol)) 均大量累積，並參與滲透保護與 ROS 的清除 (Saddique *et al*. 2020)。蛋白體資料亦顯示，植物乾旱逆境下，其熱休克蛋白質、SOD 及 APX 等抗氧化相關蛋白質之上調，有助於減輕膜脂過氧化與光合抑制 (Goche *et al*. 2025)。蛋白質體與脂質體學 (lipidomics) 研究顯示，在乾旱下磷脂 (phospholipids) 比例上升、三酸甘油脂 (triacylglycerols) 降低，可維持細胞膜流動性與訊號傳導效率 (Liu *et al*. 2021)。同時，離子體研究發現鉀、磷及鋅等離子濃度與氣孔導度及抗氧化酵素活性呈正相關，顯示其在乾旱下扮演離子穩定器角色。此外，單細胞轉錄體學 (single-cell transcriptomics) 研究指出，乾旱逆境下特定細胞 (如保衛細胞或根尖細胞) 的分子多樣性與調控網絡表現差異顯著，證明細胞型特異性 (cell-type specificity) 為耐旱關鍵 (Netla *et al*. 2023; Sonowal *et al*. 2025)。

儘管目前多體學為植物乾旱反應研究提供新的契機，但其在資料處理與跨維度的整合仍面臨挑戰。首先，不同維度體學間的表現時間延遲效應與資料異質性，使得個體學間因果關係的預測更為複雜化 (Fukushima *et al*. 2009; Hasin *et al*. 2017)。其次，植物乾旱反應具有顯著的基因型 × 環境交互作用，造成不同族群間難以建立穩定模型 (Velten & Stegle 2023)。同時，現有多體學研究資料多為室內或溫室條件下所得結果，可能與實際田間表現的情形存有差距 (Fukushima *et al*. 2009; Hasin *et al*. 2017; Sonowal *et al*. 2025)。未來，或可整合下列策略，予以克服上述課題：(1) 整合 AI (artificial intelligence) 輔助多體學分析：結合機器學習與深度網絡 (CNN (convolutional neural network), GNN (graph neural network)) 模型，以辨識非線性基因 – 環境關係；(2) 時空層級體學分析 (spatiotemporal omics)：利用空間轉錄體學 (spatial transcriptomics) 追蹤抗旱細胞群動態；(3) 表型體 - 多體學聯結：結合高通量影像與組學資料，建立基因型 – 表型 – 環境 (G-P-E) 關聯模型；(4) 資料標準化與共

享：統一格式化標註與跨平臺數據標準，促進多研究間比較與再利用 (Goche *et al*. 2025; Sonowal *et al*. 2025)。

耐旱性育種應用現況：分子標誌輔助選拔與品種開發實例

為因應日益頻繁乾旱與匱乏水資源的挑戰，水稻耐旱育種已成為全球水稻生產穩定性的重要課題。透過前述水稻耐旱數量基因座定位與功能基因的選殖，研究者得以發現與耐旱關鍵 QTLs 與候選基因，並結合分子標記輔助選拔 (marker-assisted selection; MAS) 技術，有效導入至現行水稻商業品種。然而，多數耐旱育種相關計畫，有效耐旱品種開發的成功率仍偏低，主要受限於耐旱基因來源、多變的環境條件及目標導入品種的複雜遺傳背景 (Dixit *et al*. 2017; Barik *et al*. 2019; Singh *et al*. 2020; Kumar *et al*. 2021)。

國際耐旱品種開發的成功案例

IRRI 在過去十餘年積極導入耐旱 QTLs 堆疊策略，應用多個耐旱效益 QTLs，導入傳統高產品種，以提升其在乾旱逆境下的穩定產量表現。其中，qDTY 系列 QTLs 尤具代表性：(1) 育種家已成功將 $qDTY_{9.1}$ 導入至大面積栽培之高產品種—‘IR64’，實現對乾旱逆境的適應力提升 (Singh *et al*. 2016)；(2) Shamsudin *et al*. (2016) 成功透過分子輔助育種技術將 3 個耐旱 QTLs ($qDTY_{2.2}$、$qDTY_{3.1}$ 及 $qDTY_{12.1}$) 導入馬來西亞水稻品種 ‘MR219’，有效強化其在乾旱條件下的穩定生產力 (Shamsudin *et al*. 2016)；Dixit *et al*. (2017) 亦堆疊 3 個 QTLs ($qDTY_{3.1}$、$qDTY_{6.1}$ 及 $qDTY_{6.2}$)，開發於乾旱條件下具有高產特性的水稻品種—‘TDK1’ (Dixit *et al*. 2017)。

過去商用品種的應用限制與未來需求

早期推薦於灌溉田種植的 ‘Swarna’、‘Sambamahsuri’ 及 ‘IR36’ 等大多數高產品種，已被應用於耐旱育種計畫作為耐旱性狀的接收親本。尤其，當農民於降雨生態系統中種植這些品種時，若遭遇頻繁乾旱期，將因這些高產品種不具耐旱的特性，而造成嚴重的產量損失 (Hao *et al*. 2018)。然而，因過去未能針對乾旱適應性，建立有效測量與篩選方法，導致高產品種在實際耐旱環境中表現落差的主因之一。未來耐候水稻育種的核心挑戰，將不僅侷限於提升產量，更需聚焦於高產與耐旱性之協同育成，同時兼顧其對氣候變異與多重逆境 (multi-stress) 條件的適應能力。這包括：(1) 廣域適應性育種：針對不同氣候區域，建立多環境試驗體系，確保所選材料在多樣場域仍具穩定表現；(2) 應用高通量表型分析技術：建立客觀、量化且田間可操作的乾旱耐性指標，提升耐旱性狀的量測效率與選拔精準性；(3) 跨維度資料整合應用：結合 QTLs、候選基因、miRNA 及表型體數據，透過 AI 分析與機器學習模型發展新一代選拔策略，突破傳統單性狀選拔限制。

結語

水稻耐旱改良育種必須同時解決耐旱機制複雜、耐旱基因受環境強制約及耐旱外表型選拔指標不足等三項關鍵瓶頸。在耐旱機制部分，現學界已完成形態 – 生理 – 生化反應 (如：LR、RWC、WUE、量子產率、滲透調節及抗氧化代謝) 等相關定義，藉以提供可量化的田間耐旱性狀評估參考。同時，現已採勘耐旱 QTLs ($qDTY_{1.1/2.2/3.1/12.1}$ 與 $qDTR_{8}$、$qDLR_{8.1}$、$qLR_{9.1}$ 等)、已選殖耐旱功能基因 (*OsNAC5*、*OsCPK9*、*LEA*、*OsDREB2A*/*2B*、*OsDRAP1*、*OsPYL*/*RCAR5*、*EcNAC67*、*CDPK7*、*CIPK03*/*12*、*WRKY*、*OsITPK2*) 及 miRNA (miR393、miR398、miR160/167、DST-amiRNA 等)，這些成果則已建構分子育種上可操作的遺傳工具。另一方面，育種實務上分子輔助選育/分子輔助回交育種 (MAS/MABC) 與 QTLs 堆疊等策略，已被證實可在多環境背景下，有效提升耐旱的遺傳增益。但現實育種工作上，仍受限於上開 QTLs 區間過大、連鎖累贅及基改作物安全疑慮與基因型 – 環境 (G × E) 交互作用等限制。未來育種及產業應用策略面上，進一步可採高通量表型體分析平臺 (田間多維度偵測器分析平臺) 將核心生理性狀標準化，建立跨環境與

多維度的穩定耐旱選拔指標。同時，利用測序基因分型 (genotyping by sequencing; GBS) 策略，堆疊導入多種不同機制且來自自然種原的耐旱基因型，同步完成重組體之前景、重組及背景的基因型選拔，續進行多區域實驗點與多年期外表型田間試驗，進而建立環境共變量 (environmental covariance) 與實際耐旱反應的 AI 分析模型，得以整合基因型 – 外表型 – 環境 (G × P × E) 可解釋之 AI 與基因組選拔框架，量化單基因與組合效應，及其對產量構成與環境資源分配的貢獻。展望未來，仍需要整合多體學與表型體學 (multi-omics and phenomics integration) 的研究正重新定義植物乾旱適應的理論基礎。透過系統層級資料整合，科學家得以揭示分子調控 – 代謝流 – 生理表型的連續動態過程，並辨識具預測潛力的關鍵代謝節點與調控轉錄因子網絡，並將所得的數據進行有效的整合，方有機會建立作物設計基礎 (crop design based) 的數位育種及智慧管理平臺，以因應未來更形嚴峻之氣候變遷的挑戰。

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Advances in Research on Drought Resistance Mechanisms and Molecular Breeding Strategies in Rice

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Abstract

Lin, D. G., Y. L. Lin, Y. W. Wang, S. Y. Lin, C. B. Li, W. C. Wen, J. Y. Wang, C. S. Wang, and S. Chen. 2026. Advances in research on drought resistance mechanisms and molecular breeding strategies in rice. J. Taiwan Agric. Res. 75(1):51–72.

Rice, a water-demanding staple crop, faces escalating risks from climate change and freshwater scarcity, making improved drought resilience essential for food security and sustainable agriculture. This review synthesizes physiological, biochemical, and molecular responses of rice to drought, emphasizing actionable avenues for breeding. Core adaptive processes include osmotic adjustment, reactive oxygen species (ROS) detoxification, stomatal regulation, and remodeling of root system architecture. Advances from quantitative trait locus (QTL) mapping and gene cloning have identified drought-associated regions and genes- such as the qDTY series (e.g., $qDTY_{1.1}$), $qDTR_8$, $qDLR_{8.1}$, and candidate genes including *OsNAC5*, *OsCPK9*, and *LEA* proteins, that provide valuable genetic resources for marker-assisted improvement. Small-RNA regulation constitutes an additional control layer: over 30 drought-responsive microRNAs have been reported, with at least 19 showing defined functions; exemplars include miR393, miR398, miR160, miR167, and DST-amiRNA, which modulate auxin signaling, antioxidative capacity, and stomatal dynamics. On the application front, marker-assisted selection and QTL pyramid have delivered drought-tolerant lines and cultivars. Nevertheless, field expression and precise quantification of drought-related traits remain major bottlenecks that constrain genetic gain. Future progress will hinge on integrating high throughput phenomics with genomic and epigenomic information and contextual environmental data to build multi-level regulatory networks and robust, multidimensional selection indices. Such integrative pipelines are expected to accelerate the development and deployment of drought-resilient rice varieties, advancing the intelligent and sustainable transformation of crop production systems.

Key words: Rice, Drought tolerance, Quantitative trait locus (QTL), Candidate gene, Molecular breeding.

Received: September 9, 2025; Accepted: November 14, 2025.

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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):73–85 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0004

專題論述

農用無人機，農業多元應用之現代化工具及未來展望

陳怡菁[1] 楊純明[2,*]

摘要

陳怡菁、楊純明。2026。農用無人機，農業多元應用之現代化工具及未來展望。台灣農業研究 75(1):73–85。

農耕觀念的更新與科技的進步，帶動了農業經營管理的精進與創新，從糧食生產、農產品行銷、直至農業環境保護及生態維護的思維與作法皆隨著時代脈動往前進步，期望人類能夠過著豐衣足食的幸福生活，也讓人生活出亮麗的生命色彩。農用無人機在此時橫空出世，不僅走在當前農業的產業前沿，更成為未來農業蓬勃發展的空中人工智慧助手，從單一作業機具進化為農業資訊/數據平台、逆境/災害偵 (監) 測耳目、防疫防災救援樞紐、乃至於農業淨零與碳管理的多功能工具。臺灣農用無人機在農耕體系上的引用與利用，逐漸從早期稻作栽培的噴藥施肥朝向多元的應用方向，希望未來可以結合各種感測裝置、先進科技、人工智慧、物聯網及客製化作業系統等元素，廣泛應用於進一步提升農糧生產的質量、農業經營管理的效能及應對氣候變遷與農業淨零的利器，提供數據化、多目標、高效益及低風險的多特色解決方案，貢獻於農業永續願景的實現。本文整合相關訊息與情境，描述了農用無人機的優勢與挑戰，寄望農業從業人員高瞻遠矚洞察機會所在，農業利害關係人找出產業發展利基，通力合作互利共贏迎向農業的新紀元。

關鍵詞：無人機、農用無人機、農業領域應用、精準農業、智慧農業。

前言

無人機 (drones) 或稱無人飛機、無人飛行載具 (unmanned aerial vehicles; UAVs)、無人飛行器系統 (unmanned aircraft systems; UASs)，係指無需人員直接駕駛而可由遠端或自動控制系統操控的飛行器。無人機通常由包含機體結構/飛行平台 (如固定翼飛機、單或雙旋翼直升機、多旋翼及混合型設計等)、遙控系統 (如遠端操作、預設航線及人工智慧 (artificial intelligence; AI) 自主飛行等)、動力飛行系統 (如電動馬達、燃油引擎、油電混合及太陽能等)、通訊導航設備 (如全球定位系統 (global positioning system; GPS) 與慣性導航儀等) 及感測裝置 (如可見光 (red-green-blue; RGB)、多光譜 (multispectral)、高光譜 (hyperspectral)、雷達 (radar)、光學雷達 (簡稱光達，light detection and ranging; LiDAR)、熱紅外線影像儀 (簡稱熱像儀，thermal infrared imager) 及超音波 (ultrasonic) 等) 等組構而成，牽涉續航力、載重力、偵測力、酬載容量、噪音、環保、維護及成本 (Zhang & Kovacs 2012; Esposito *et al*. 2021; Krishna 2021; Velusamy *et al*. 2022; Zhang *et al*. 2022; Guebsi *et al*. 2024)。

大致上，無人機依照研發目的或使用需求而有不同的外型、功能及規格設計，安裝特定的系統，亦隨著科技的發展在飛航距離、飛行控制、起飛海拔、資料傳輸及任務酬載上進步神速。以偵察/監測/勘查功能來說，諸如鏡頭數量、感光元件、像素、幀率 (畫面更新頻率，frame per second; fps)、高倍解析度、焦距及拍照與錄影等規格在近年皆有非常大的精進。如今，像素數目已提升至以億為單位，幀率已增至 100 fps 以上，相片/影片解析度高達 4K

投稿日期：2025 年 7 月 4 日；接受日期：2025 年 11 月 27 日。
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或甚至 8K 超高清品質，空拍靈活度可達 360° 水平旋轉與 70° 以上仰角，幾乎可全天拍攝、全向避障及自主剎車等，具有相當高的飛行安全性。

一般來說，無人機使用遙控、導引或自動駕駛系統來控制飛行，利用內建或外掛的感測裝置及資通訊系統來偵察/監測/勘查地景地物地形，也可搭載置物箱/櫃/桶/架進行特殊用途酬載，如科學研究、勘災救援、軍事打擊、能源採礦、物流運輸、地理測繪、建設建築、商務活動、休閒娛樂及農業植保等不同用途。隨著科技的不斷創新與功能的更新，精密高階的無人機可以從事各種的航空太空任務，或裝配了攻擊性武器成為無人戰鬥航太載具 (unmanned combat air vehicle; UCAV)、無人作戰飛機 (combat drone or war drone)，彰顯出了無人機的無限應用潛力。尤其近年來已發展出無人機蜂群 (drone swarms) 態樣，以數十、數百或成千成群的蜂群模式進行集團式的作業，發揮驚人的工作能量與效果。

在農業應用方面，無人機常被稱為農用(業) 無人機 (agricultural UAV 或 agricultural drone) 或農用植保機，除了基本的飛行、遙控及導航等組件之外，主要結合了感測裝置與資通訊科技、自動化技術、地理資訊系統 (geographic information system; GIS)、GPS 及酬載設備等 (5 大基本組件整理概如圖 1)，

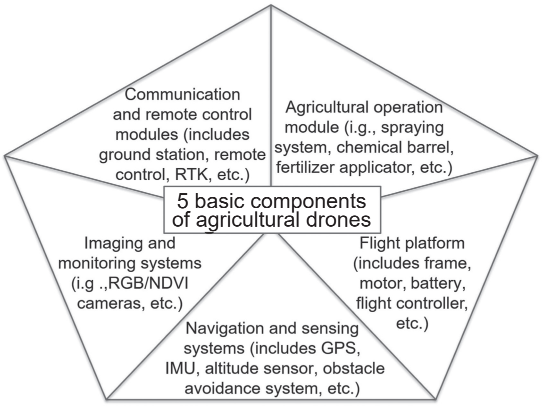

圖 1. 農用無人機之 5 大基本組件。

Fig. 1. The five basic components of agricultural drone.

以從事偵察、監測、勘查、噴灑、施肥及灑播種植等功用，專門於農業管理與生產操作相關的多元應用，據以提升農業經營的效率、精準度及永續性 (Zhang & Kovacs 2012; Yang *et al*. 2022; Zhang *et al*. 2022; Yang *et al*. 2025a, 2025b)。常見 (用) 的農業生產作業酬載模組，一般會具有藥劑箱、肥料桶、水箱或種子盒，液體噴灑系統會有幫浦與噴嘴，粒狀肥料或種子則有螺旋器或離心盤。我國農政部門 (農業部/農糧署) 亦將農用無人機視為屬於『智慧農業設備』之一，根據 2024 年公布的『省工高效及碳匯農機補助實施計畫』，凡具有農保或農業經營相關證明者得以提出農用無人機/農噴無人機補助申請，以減輕農民、農民團體 (如合作社、產銷班) 及農企業等成本負擔，減少因長期農噴帶來病症與意外傷亡，藉此擴大農業生產上之應用。

基本上，申請者須符合以下 4 項補助申請條件：(1) 已取得交通部民用航空局 (以下簡稱民航局) 核發的『遙控無人機高級專業操作證』。(2) 持有農業部動植物防疫檢疫署 (以下簡稱防檢署) 核發的『空中施作技術類別農藥代噴技術人員證書』。(3) 完成『空中施作類別農藥代噴業者』登記。(4) 所屬團體須具備法人資格，並取得民航局核發的『遙控無人機能力審查核准證明』(可進行投擲或噴灑作業與裝載危險物品)。詳細來說，依照民航局《民用航空法》〈遙控無人機專章〉的規定，無人機操作者需要參與防檢署辦理之農藥代噴人員訓練課程及取得證照，再通過民航局無人機操作證的評鑑與證照。施藥作業除了代噴人員需要取得相關證照、事先申請外，3 日內還要上傳施藥紀錄及航跡到防檢署的無人機代噴管理系統。又根據〈遙控無人機專章〉規定，只有具法人與其會員身分才能執行農藥噴灑、空拍及調查等行為，操作前必須向縣市政府申請，且操作者須領有專業操作證才能執行任務。所以，以無人機施灑農藥除了需要符合民航局的飛行安全規定外，施灑農藥的行為也要受到防檢署規範，所有無人機代噴操作者必須同時擁有防檢署之農藥代噴人員專業空中施作證照及領有民航局所發放的無人機高級專業操作證 (G1、G2 等級) 才能合法代噴。

有鑒於農用無人機具備的各種加值應用條件，幾乎可以滿足代噴業者與農民的需要，本文乃特地蒐集與整理有關農用無人機在農業領域應用上的相關訊息/資料，期以提供農業主事者在政策與技術發展上的規劃依據，並作為基層農事操作者 (如農民、青農、產銷班、農場、合作社及農耕管理者) 在實務作業上的利用參考，同時期待科研機構未來在功能與系統整合上有更多的研究開發，使農用無人機無論現在或未來皆成為農業各領域眾多問題的有效解決法寶。

農用無人機之類型、使用效果及操作安全

相較於傳統飛機，農用無人機具有操作成本低、運用彈性大及支援裝備少等特色，目前世界上許多工業發達的國家莫不積極的開發與利用各種用途的機種 (型)。因此，市場上的農用無人機種類甚多，分別在體型、性能、重量酬載、飛行高度、作業時間及飛航距離等有不等規格可供選用。

農用無人機之類型

無人機依機體結構可概分為固定翼無人飛機、無人直升機 (單旋翼或單軸雙旋翼)、無人多旋翼機及混合式或複式無人機 4 種類型，各類型在起降靈活度、作業面積、飛行範圍、續航力、速度及精準度上各具優缺點，因此在應用上需要依照客製化的需求而異。其中，固定翼無人飛機外觀似於單一剛性機翼的小型飛機，主要為了大面積長時間飛行而設計，非常適合繪製與測量大片農田。雖然機動性略遜於多旋翼無人機，其長時間飛行優點適用於繪製、勘查及監測大面積農田，並測繪詳細的正射影像地圖 (orthomosaic maps) 與三維 (three-dimensional; 3D) 地形模型。無人直升機 (單旋翼或多旋翼無人機) 與無人多旋翼機 (具有多個旋翼，通常為 4–8 個)，具有垂直起降與空中懸停功能，由於能耗較高而飛行時間較短、每次飛行覆蓋範圍有限，惟具有機動性較強、穩定性較高、用途較廣及易於操作與部署等優勢。且由於能夠懸停在原地，而適合執行精確的任務 (如目標物詳細檢查、農田局部監測、資材噴灑及針對性用途項目等)。混合式或複式無人機則結合了固定翼與多旋翼的特點，兼具兩種類型無人機的優點與應用選項。但是，組件較為複雜、機身較重及價格較昂貴，也需要更多維護。綜上所知，臺灣農田規劃面積小，固定翼無人飛機不適合應用於農噴作業 (不能懸停)，僅能適用無人直升機 (單旋翼) 與無人多旋翼機 2 種，其中無人直升機因操作技術門檻高及無人多旋翼機操作簡易，因此目前臺灣農噴、施肥及種植多以無人多旋翼機為主。惟目前民航局規定同一人僅能操控 1 架無人機，而無人機蜂群僅能在表演及軍事用途。未來農用無人機除了本身軟硬體的更新進步之外，將整合 AI、物聯網 (internet of things; IoT) 及先進感測系統，實現即時資料處理、決策及自主操作來執行任務，減少對人工干預的需求。

農用無人機之操作安全

農用無人機的作業效率、低勞動強度及高安全性，徹底改變了現在慣行的農耕作業模式。為了提升農用無人機的使用效果與操作安全，需要注意至少 5 項重點，包括：(1) 起飛前、後 360° 檢查，含螺旋槳及電機 (馬達) 是否有裂痕、各螺旋槳旋轉方向性是否正確、機身與酬載是否穩定、機臂是否鎖固、飛控系統、GPS 是否穩定、電池電量、藥桶及全系統 (遙控器與無人機訊號鏈路) 是否正常等；(2) 目標區航線規劃，如利用無人機配備 App 軟體設定航線、施作區塊、飛航高度及飛行速度等；(3) 飛行操作，採用手動或自動飛行，設定起飛、降落、噴灑、避障及地形追蹤等；(4) 安全距離，即機體與作業人員、地面、電線及建物間之距離 (至少 20 m 以上)，田間執行噴藥飛行高度為作物植冠上方 3–4 m；(5) 當時氣象狀況，如風速 (< 2 級)、陣風、降雨、濕度、氣溫及能見度等影響飛行安全與作業品質的因子；(6) 維修保養，可區分飛行前、後檢查、基礎保養、深度保養及年度檢測等 4 大項 (Rejeb *et al*. 2022; Felkers *et al*. 2024; Advexure, https://advexure.com/blogs/news/spray-drones-vs-traditional-crop-spraying-

which-is-more-effective; Investigate Midwest, https://investigatemidwest.org/2025/01/08/as-the-agricultural-drone-industry-takes-off-federal-regulators-struggle-to-keep-up/; Silveus Crop Insurance, https://silveuscropins.com/drones-in-agriculture-the-future-or-over-rated/)。對於基層農事操作者來說，一般可檢查飛控、電池、螺旋槳、GPS、實時動態 (real-time kinematic; RTK)、感測器等功能狀態，清潔機體、處理藥劑與肥料殘留、液體輸送管線及檢視螺絲與鏽蝕等。

農用無人機在當前農業領域上之應用

農用無人機在農業領域上的多元應用，為農業從業人員與農企業提供了提高產量與減少作物損害的新方法，無論在土壤肥力、種子撒播、灌溉、除草、病蟲害防治、糧食產量估測與預測，以及資源與人力節省等項目，皆彰顯出明顯的經濟優勢，乃至於延伸至防減災、勘災及救災等利用 (Olson & Anderson 2021; Yang *et al*. 2022)。自從精準農業 (precision agriculture; PA) 倡議於 1980 年代以來，農用無人機更已成為農業產業的變革者，處於這項大變革的前沿，成為務實的觸媒及有效工具 (Yang *et al*. 2022; Yang *et al*. 2025a, 2025b)。各種配備了先進感測器、相機/攝影機與噴灑系統的農用無人機，使基層農事操作者能夠以高精準度技術來監測、分析及處理目標農作物，讓精準農業的曙光進一步擴散放大 (Primicerio *et al*. 2012; Mulla 2013; Zarco-Tejada *et al*. 2014; Kumar *et al*. 2023)。於是，農耕操作關注的即時作物與土壤健康監測、精準施用農藥與肥料、減少化學品使用與對環境負面衝擊、以及提高作物產量與資源效率等 4 大訴求，得以在農用無人機的協同操作下充分實現。在邁向智慧農業的現在，農用無人機仍然是有力的助手，在感測、偵察、調度及施作的整合扮演樞紐角色 (Hunt & Daughtry 2018; Kumar *et al*. 2023)。

目前在臺灣由於農用無人機相關設備有一定的建置成本，操作使用需要成熟的技巧，操作人員需要具備證照，又有作業飛行空域使用申請規定、農耕目標區破碎地形等之使用限制，因此在農業生產應用上需要選擇較大栽培面積的作物種類以符合成本效益。於是，農用無人機現在大多著重於田間栽培如水稻、玉米及甘藷等大宗糧食作物，或如荔枝、芒果、文旦、柑橘、鳳梨及香蕉等大栽植範圍果樹作物，或如茶樹、甘蔗、香料、藥草、菸草及花卉等經濟作物為主，最近也開始擴及如高麗菜、胡蘿蔔、洋蔥及青花菜等蔬菜類作物。在各項農用無人機的操作型態中，臺灣現行的應用以噴灑液態農藥 (如殺蟲劑、殺菌劑、除草劑及生長調節劑等)、肥料 (如化學肥料、有機肥料及緩釋性肥料等) 最普遍，注重劑量與施作範圍的精準控制，以有效節省成本及提高人員與作物安全。其次，尚有種子的播種/撒佈，如直播水稻、休耕田油菜、田菁等綠肥植物、坡地水土保持多年生草類及果園補播等。某些特定機構、科研單位及政府部門另有其他的應用，如水稻、蔬菜、果園及茶園等之病蟲草害、逆境 (如缺水與缺肥)、或生長與產量判讀等，以及農業氣象有關的災損調查 (如颱風、豪雨及寒潮)、農地的濫墾盜伐、農糧生產計畫、灌溉設計及生產自動化作業路徑規劃等。這些逆境與災害所造成的農作物生產損失，也可以供作農業保險或政府補助的審核依據/佐證，並實現環境、社會及管治 (Environmental, Social and Governance; ESG) 的永續農業政策目標。這些多元用途在未來應用上皆可繼續強化其深度與廣度，增加應用領域，以增進農用無人機在農業各層面上之使用價值。特別在農業持續面臨氣候變遷、資源稀缺、農業淨零及智慧轉型等多項挑戰下，創新又務實的解決方案更顯重要與需要。

農業領域之應用類別

由於農用無人機可以融合農耕 (業) 運作所需的相關技術及現代化科技，因此非但農耕效率與營農效益可以獲得提升，農業的永續性願景也能夠達成，並且透過 AI 數據驅動的解析洞察能力，可讓農耕操作者與管理人員做出合適的決策判斷方案。農用無人機的選擇，通常依照應用方向與目的而有不同的機型與酬載

系統考量，偏向客製化的需求導向。綜合以上，本文謹將其在當前農業領域上之應用概分為 6 大類 (表 1)，並予以依序簡要說明作為使用者利用之參採。

農情監測──農作物偵察、監測及檢測

農情監測的農用無人機常俗稱為『空拍機』或『航拍機』，一般以內建或外掛的相機/攝影機/感測器進行目標農業生產地區的地景地物地形拍攝、記錄，可使用於偵察/監測/檢測農作物生長發育、農作物與土壤即時健康狀況、作物相與面積分布、特殊情境造成地景地物變化等即時或長期改變、資源資材使用、逆境狀態 (如病蟲草害、缺水乾旱、營養缺乏等)，以及後續的變化與變遷追蹤 (Zhang & Kovacs 2012; Yang *et al*. 2025a, 2025b)。這些掛載之 RGB、多光譜、高光譜、熱像儀及 LiDAR 等感測裝置，經由網路通訊直播、顯示、錄影或數位儲存可從事農情監測的酬載作業，作為現況因應分析、判斷及決策的根據，或接續的研析與綜合處理等工作。在當前氣候快速變遷的大環境下，地情與農情變化加大，無需專用起降場地與具高機動性的農用無人機提供了遠距、大面積、快速、即時及自動的監測功能，也可定期巡航建立生產過程的基礎 (本) 農情資訊。如此，取代人力或手動操作，可達到節省時間、勞力及成本的效果，並可迅速取得目標訊息做出及時與即時因應對策，誠為農業從事人員一個有用的農事好夥伴。

表 1.　無人機在當前農業領域上之 6 大應用類別。

Table 1.　Six major application categories of drones in the current agricultural field.

Category	Application
1	Agricultural surveillance- Crop scouting, monitoring and testing
2	Precision agriculture and smart agriculture
3	Aerial spraying, seed broadcasting and planting
4	Livestock surveillance and feeding
5	On-site mapping and 3-dimensional modeling
6	Production operations, resource management, and yield estimation and prediction

精準農業與智慧農業

農用無人機有助於繪製農田土壤變異性與農作物活力之時間序列變化圖示，在 GPS 與 GIS 協作及搭配 IoT 無線網路連結大數據 (相關資料庫) 與決策支援系統，可自動或人為指引合適農用機械與農業機器人進行對應之精準與智慧化管理操作，落實特定地點管理 (site-specific management) 精神與精準智慧化境界 (Yang *et al*. 2022; Kumar *et al*. 2023; Agrawal & Arafat 2024; Guebsi *et al*. 2024; Yang *et al*. 2025a, 2025b)。無論係自然環境下發生的病害、蟲害、雜草干擾、高溫或低溫、缺水或淹水、礦物元素不足或過度、毒害物質或重金屬等逆境，或是極端天氣事件的高強度傷害，皆可以農用無人機從事逆境與災損的偵察/監測/勘查，給予適時適地的精準處理，兼顧降低時間、人力、資材的花費成本，又可減少傳統農耕施作人員暴露在藥劑中而提升安全性、提高植保效果。

又從防災救援的角度，前述農情監測的歷史資料與即時偵察結果，除了預警預防之外，也讓農用無人機發揮突發性或臨時性的災情監控、救助、支援、防護等功能。因為農用無人機能有效規避地面障礙、阻撓，快速準確的抵達指定災害發生現場，利用資通訊設備與技術將實情信息回傳處理中心，給予指揮人員做出決策的依據，或直接攜帶相關器材物品至現地達到最快的及時救災救援。在極端天氣發生頻率與強度增加的當下，許多偏遠的受災地區以傳統人力巡檢甚為困難，農用無人機的低成本、強靈活度、高安全性及廣視角等特性，加上受自然環境及地形地物較小影響的彈性，成為最佳的防災救援首選。未來隨著感測技術與輕 (微) 型機型的日益進步，雖然未如露地栽培的廣泛範圍，農用無人機可以延伸擴大在設施農業上的各種應用，如遮陰棚、網室、溫室、垂直農場型設施、植物工廠及智慧型設施等，進行精準智慧化的操作與細部監測。在考量設施高度、架構、作物相及空間配置等條件下，導入最適大小機型以利於航線與任務之規劃，然後以掛載熱像儀或氣體感測器掃描設施內部熱點、氣流分布或濕度差異，串接設施內部微

氣象設備之感測數據，輔助進行設施環控 (如溫差、濕差、風差) 之監測、分布評估及調節。

空中噴灑與播撒/種植

利用附掛裝載農藥、肥料、水箱甚至於種子之農用無人機，可在空中噴灑殺蟲劑、殺菌劑、除草劑及生長調節劑等農藥從事田間生產管理，或播撒肥料、水、種子等調節農作物生長資材投入，快速而有效地做出情境處理，尤其適用於曳引機或灌溉無法到達的地區 (Marzuki *et al*. 2021; Yang *et al*. 2022; Kumar *et al*. 2023; Wang *et al*. 2023; Kumar & Sriram 2024; Agricultural Chemicals Research Institute, https://www.acri.gov.tw/Uploads/Item/9525e5d2-4a15-4439-80ca-813c062d8786.pdf; Yang *et al*. 2025a)。對於農田的灌溉決策，除了掛載之多光譜、高光譜、LiDAR 及超音波等感測器所得知的數據之外，可綜合熱像儀偵測土壤及植體間之熱溫變化與差異，予以即時辨識缺水區域並解決灌溉問題。又配合先進微霧噴嘴的開發使用，精確控制每一滴液滴的大小與噴霧模式，附掛於農用無人機依照特定需求客製化的液滴噴灑方案，可以精細霧化提高覆蓋率、以各種液滴尺寸調整適應不同應用、以合適噴霧模式予適用不同作物類型與生長階段，或以自動化精準控制來減少漂移與脫靶現象，從而提高噴灑效果及最大限度地減少了浪費與對環境的影響。而利用新近開發之特殊農用無人機，可將種子莢直接投擲於土壤，特別是在重新造林、災後重建或覆蓋作物的情況下，將可有效的執行農田與林區播種或補植工作。

牲畜監測與豢 (飼) 養

以農用無人機偵察/監測大面積牧場，尤其是偏遠地區的牧場，將可協助識別動物的活動與分布、水源的供應與可用性，以及潛在的災害威脅等，大大提升牧場建置規劃及營運管理成效。對於開放性大片牧場，牧草的生長與分布不一，透過農用無人機的定期巡航監測提供的感測數據，可應用於實施牧草栽植與管理及豢養牲畜的移動路線調整，助益於放牧型牲畜豢養的效果 (Andrew *et al*. 2019; Burke *et al*. 2019; Alanezi *et al*. 2022; Chou & Huang 2025)。

現場測繪與三維建模

利用農用無人機掛載之感應器 (如 RGB、LiDAR、多光譜、雷達及超音波) 產生之正射影像圖、等高線圖 (contour maps) 及數位高程模型 (digital elevation models; DEMs) 等圖資，可使用於土壤測繪、土地整平、灌排水規劃、場地設計及進出物流等各種用途 (Aasen *et al*. 2015; Tripicchio *et al*. 2015; Milella *et al*. 2019; Potena *et al*. 2019; Liu *et al*. 2024)。除了可大幅縮短傳統人力的工作時間與增進時效，農用無人機鏡頭抓取的數據呈現出的實地、實時、實景等圖像通常更為精確、客觀、使用靈活，並可以 GIS 堆疊多重信 (訊) 息，能滿足不同調查與測繪的需求。例如，將不同感測數據建立田區 2D 或 3D 地景圖、地形圖，呈現所偵測病害、蟲害、雜草、缺水、淹水，或營養不良等造成的異常情形，可輔助精準農業及智慧農業的決策，進行區塊耕犁、施肥、噴藥、除草、灌溉或其他因應作業，將可獲得精準智慧化的管理績效。

生產作業、資源管理及產量估測與預測

藉由無人機建立的歷史數據與即時圖資、天氣模式及當前作物與土壤健康狀況，可協助進行定期或特定的田間環境/狀況調查、地理測繪及生產評估 (Lelong *et al*. 2008; Zhang & Kovacs 2012; Bendig *et al*. 2014; Sankaran *et al*. 2015; Holman *et al*. 2016) 任務。這些無論是土壤、水源、農作物、雜草、病蟲害、災情，或地形地物地景等數據，可接續其他如科研、勘災、搶險、精準智慧化農耕操作及資源管理 (如土壤、水源、資材等) 等具體應用。尤其在需要繁複程序的農糧生產作業，以農用無人機取得諸般數據結合 IoT 的大數據與決策系統，將可游刃有餘的落實於從耕地、播種、施肥、噴藥、生長追蹤、直至生產估測與預測等環節，乃至於採收後的物流運輸。此外，從農作物成熟度與健康狀況的數據評估與追蹤，可確定最佳收穫物採收時間，以確保最佳的品質與產量 (Yang *et al*. 2025b)。也可有效地安排勞動力與設備、減少農機作業停機時間及成本花費，進而提高了農耕獲利能力。這些數據若進入模式分析來估測與預測當季的生產質量，將

可提前進行糧食供需評估、產業輔導及政策措施布局，提升了農業施政的效能與效益。而相對於傳統物流方式，利用農用無人機來空運，更具有避免交通堵塞、規避危險地形及快捷高效安全運輸等明顯優勢。尤其在山區或偏遠地區，農用無人機的物流運輸將可節省更多時間與成本，減少對有限人力資源的依賴。

總之，農用無人機在以上 6 大類農業領域上的應用，將可裝備農業從業人員成為善用現代科技的『新時代農民』，並賦予農村社區成為大有能力的『高效率高獲利新農村』。農用無人機相關技術創造出的顯著經濟影響是難以想像的，其與農業的融合不僅僅是一項技術進步，也是農村與農企業大發展的經濟催化劑。這些經濟貢獻，包括了提高農業生產力、節省肥料及農藥投入成本、技術服務提供新收入來源、投資農村基礎設施以支援農用無人機相關技術精進，以及整體活動所產生連鎖反應，預料將可振興農村社區的經濟成長。因此，可以預期必將給農業帶來深遠又厚重的影響，不但解決長期存在的農業生產挑戰，亦創造新的農業產銷鏈與在農業領域上的就業機會，更將吸引年輕世代從事農業、增強農產品在全球市場的競爭力，使農業真正成為可實踐、有利潤及永續的農產業綜合生態系統。

農業領域之應用挑戰

儘管如此，當前農用無人機的應用仍存在一些技術與實務挑戰，有待各方予以克服，如高初始成本、複雜技術、操作人員熟練程度及飛航與空域限制等，也受到強風暴雨等極端天氣的飛行阻礙。再者，由於電池壽命與有效載荷能力有限 (尤其是多旋翼無人機)，限制了無人機運行時間與範圍，且頻繁充電及攜帶重型感測器或大量資材的容量也會降低運作效率。惟在權衡初始投資與長期投資回報 (如減少化學品使用、節約用水、改善農作物與土壤健康等)、環境效益及創新農業實踐的潛力，農用無人機操作確實對整體農業生態產生了積極與正面的美好藍圖。另外，本文特別要強調控管噴施化學品外溢的重要性，對於農田面積狹小的臺灣，噴施化學品如飄溢至非標的區的鄰田將可能造成糾紛與困擾。

因此，未來農政部門如欲促進與推廣農業人工智慧及無人機的整合應用，需要有宏觀遠見及長期的進程規劃，加上有利的政策與經濟誘因，以跨越各方挑戰。尤其在與農民溝通及農業專業人士合作的過程中，仍有需要討論及解決的許多關鍵議題，諸如：(1) 法規的滾動調修與遵守、(2) 空域的劃設與管理、(3) 農場的成本融資與輔導、(4) 操作人員的訓練與技能精進、(5) 資料與作業系統的管理與安全維護，如此才能夠建置出農用無人機技術及產業的發展環境與市場商業模式。政府如何吸引科研機構與農企業的投入意願，以及廣大基層農事操作者的參與，並且減少衍生的資安風險、隱私保護、資料流失、被動失業及環保影響憂慮等問題，也是成功的門檻。這些樣樣事工，都需要農業相關利害關係人 (含政府、科研機構、農企業、非營利組織及基層農事操作者等) 的齊心協力攜手合作以克竟其功。

農用無人機在未來農業領域上之應用方向

在可預見未來，農用無人機將是一個整合多種技術與設備於機身的多功能多元應用的飛行載具，能夠與其他前瞻農業解決方案相結合，包括拼接衛星影像、AI、IoT、農機設備及農業機器人等，來協同執行特定或客製化的例行工作與特殊任務，創造一個全面全新的農場管理系統。舉例而言，當農用無人機資料補充衛星影像後，可實現更精確的農作物與土壤健康評估及灌溉需求，並可從空中監測牲畜群運動、行為、健康狀況及輔助自動識別受傷或生病動物，以及結合機器學習構建氣候智慧型農業 (climate-smart agriculture)。將 AI 演算法的充分利用，可使農用無人機處理收集的數據以進行現況及預測分析，使形成為數據推動型農耕 (data-driven farming)，一方面可以更高的精度預測病蟲害爆發、營養缺乏及生產質量，另方面可進行農作物表型與基因型高通量/育種研究加速育種週期，以及評估農田區塊的生長率、生物量及冠層特徵。串接合適的農機與農業機器人，農用無人機可成為指揮進行設定農耕操作的樞紐，目前民航局無人機法

規規定 1 人僅能操控 1 架，未來法規開放後可以農用無人機蜂群協調噴灑、灌溉及播種，針對性地噴灑農藥與肥料以減少化學物質的使用與環境影響。經由連線 IoT 與感測器網絡，農用無人機可進行特定性的干預與處理，以評估風暴、洪水或乾旱等災害造成的損失而應用於災害與氣候變遷應變、保險及復建 (救)，又在碳農業/農業淨零與永續性監測上發揮功能。當連結區塊鏈技術去中心化，農用無人機感測數據可確保供應鏈的透明度，並將控制與決策從集中式實體 (個人、組織或團體) 轉移到分散式網路來擴大應用 (Huang *et al*. 2009; Zhang & Kovacs 2012; Sankaran *et al*. 2015; Kamilaris & Prenafeta-Boldú 2018; Rahman *et al*. 2018; Lal 2020; Martínez *et al*. 2020; Wang *et al*. 2024)。

這樣整併不同系統組件的綜合作業方法，不僅可以提高農場生產力，而且有助於實現更永續的農業運作模式，有效應對全球氣候變遷與糧食安全的重大挑戰 (AgFunder Network Partners, https://agfundernews.com)。本文綜合以上所述要點，歸納出農業領域的 7 大未來應用方向，摘述如次。

自主協同工作無人機蜂群 (autonomous drone swarms)

未來的農業生產工作，將可以農用無人機蜂群採行分進合擊的集團式協同作業，針對同一工作場域併行執行多項任務 (如偵察、監控及噴灑等)，來提高作業效率；或以責任區劃分方式由農用無人機蜂群依照責任區塊做工，在同一時間共同執行相同的工作項目 (如播種、噴藥、施肥、灌溉、勘災、吊掛及搬運等)，來縮短執行任務所需要的時間及人力 (Virágh *et al*. 2013; Vásárhelyi *et al*. 2014; Floreano & Wood 2015; Zhou *et al*. 2020; Qamar *et al*. 2022; Weber 2024)。掛載各種微型感測器，農用無人機蜂群的定期巡航或臨時拍攝農作物生長情形與土壤狀況，將有助於進行固定或即時作物與土壤健康監測與追蹤，包括在預定場域或難以抵達處所。

支持達成人工智慧決策 (AI-powered decision support)

農用無人機未來可以取得認證 (如 beyond visual line of sight; BVLOS) 被允許在視線以外運作，如美國聯邦航空總署 (Federal Aviation Administration; FAA) 的授權，而且將獲取的資訊整合 AI 進行即時分析與自主決策，迅速地執行任務 (Tripicchio *et al*. 2015)。

與 IoT 及機器人 (robotics) 技術的集成

農用無人機未來可以串接 IoT 廣泛連接相關系統 (如空中與地面感測器、自動曳引機及農業機器人等)，並搭配大數據及決策系統的 AI 演算，使用於運作無縫智慧農場生態系統 (Agrawal & Arafat 2024)。

病蟲害預測與早期干預

農用無人機藉由IoT串接來自多個資訊來源 (如天氣、土壤、歷年模組) 的 AI 及大數據，可進行預測建模與早期病蟲害預警 (Zhang & Kovacs 2012)。例如，透過微型或小型無人機掛載之影像與光譜辨識、熱影像偵測病斑與蟲害熱點等資訊，搭配 AI 模組與機器學習模型進行病蟲害初期偵測，可預測病蟲害爆發時間與農田區塊，預先進行精準農藥噴灑處理，以早期施給對應措施來排除或減輕病蟲害之危害。

協助氣候調適

農用無人機隨著酬載軟硬體的更新精進，未來將在氣候智慧型農業中發揮關鍵作用，可協助管理耐抗逆境農作物生長，並監測不斷變化的農業生態區。也可利用於溫室氣體/二氧化碳監測、生態農藥效率追蹤等用途，成為多功性的永續智能工具 (Tripicchio *et al*. 2015; Sylvester 2018)。

區塊鏈與農作物可追溯性 (blockchain & crop traceability)

利用農用無人機收集可使用於追溯系統的數據，配合 GIS 標記影像與飛行日誌，可提高供應鏈的透明度與區塊鏈的運轉 (Chou & Huang 2025)。同時，未來將可誕生農用無人機

即服務 (drone-as-a-service; DaaS) 的產業鏈，以租賃或共享模式取代農用無人機所有權，由專業服務代替目前大多數的個別實際操作態樣。

自動化相容合規與報告 (automated compliance and reporting)

農用無人機未來可利用於綜整相關資料來自動產生監管合規報告，特別是在圖資測繪、有機產業、進出口或碳信用等農業領域 (Varanasi *et al*. 2022; Ni 2025)。例如，經由即時 3D 測繪模型與監測覆蓋農作物、碳封存/碳匯數據及生物多樣性等碳農業與永續性相關資料，將可從事於認證碳信用額度與永續性指標等工作。又由結合 quick-response code (QR code) 與 bar code 等條碼視覺辨識系統的拍攝，可利用於快速辨識不同苗盤/植株、所在位置及植栽密度與數量等，以及作為自動盤點與記錄及物流管理用途。當配合多時點影像拼接技術，可將長時間記錄累積之高解析圖資變化進行不同時差比對，可釐清設定起訖時間所造成標的物之改變。

總結以上農用無人機未來在農業領域的 7 大應用方向，再度顯示農用無人機不僅提高現代農業運作的效率、精度與永續性，未來再因為融合智慧農業與 AI 的數據分析及自動化特色，必然將會迅速增加其在農業各層面上扮演的角色，並將使得採行農用無人機整合技術的基層農事操作者成為下一世代高競爭力的科技農民 (Sundar *et al*. 2023)。

農業人工智慧 (artificial intelligence in agriculture) 與農用無人機的整合應用

AI 與智慧農業科技的蓬勃發展，除了將促使農用無人機在各項軟硬體的發展一日千里，更得以在農產業上發揮無限寬廣的應用，也漸形成所謂的『農業人工智慧』創新研究與應用方向。本文因此特別就此面向加以闡述，並以補強先前的論述。基本上，農業人工智慧係指以數據為基礎的方式支持與改進傳統農業經營的做法，同時減輕各方挑戰，綜以提高農業的經營創新、營運效率、降低成本及勞動力需求，並提高操作人員的舒適度、滿足需求考量。在農業生產實務上，農業人工智慧為基層農事操作者提供即時農作物洞察解析，藉由 AI 優化資源供需與改善農作物管理來發揮關鍵作用，協助確定哪些區域需要灌溉、施肥及噴藥等處理或其他應對作業。換言之，就是透過 AI 先進的數據分析與機器學習能力，來解析作物與土壤健康狀況、天氣模式與環境狀況，從種植、灌溉、施肥、噴藥、除草至採收提供完整而精確的各樣生產環節決策建議，產生出農作物產量估測 (算) 與預測模型。如此，將可同時增強農作物管理、降低生產成本、維護環境，延伸解決農業、食品、生物及生態系統領域遭遇的各種問題，從而優化生產與營運流程並促進了農業的永續。

所以，農業人工智慧技術在農業生產實務上至少可歸結出 7 大應用潛力，包括：(1) 作物病蟲害檢測、(2) 自動除草系統、(3) 牲畜豢養與健康監測、(4) 作物產量估測與預測分析、(5) 精準灌溉系統、(6) 無人機輔助空中監視及 (7) 農產供應鏈與需求預測。這些應用潛力的內涵一如前述，重點在於透過 AI 技術的數據運算總結出容納各相關面向的最佳因應決策，為農業從事人員提供能夠做出因應的數據驅動決策及邏輯知識，減少農業的生態足跡並適應氣候變遷與資源稀缺帶來的挑戰。相關農業部門則可經由開發農業人工智慧工具的推廣應用，協助基層農事操作者來縮短區域技術差距、提高生產力、減少損失，再輔以智能供應鏈與產銷鏈來提升生產效能與效益，建構有利潤又可持續的永續農業生產體系。

另外，未來將農業人工智慧與農用無人機的融合集成，不僅是產生一種智慧的農耕作業系統，更代表著現代農業實踐的大變革、大進步，也引領通往新農業世紀的大門。配備農業人工智慧的農用無人機在監測農作物表現、增強農作物與土壤健康、優化資源供需利用及改善整體農場管理等方面將具有無與倫比的能力，可進一步發揮精準智慧化的功效，更改變了現代農業的格局，提供了超越單純效率的眾多好處。因此，利用農業人工智慧提供的複雜數據分析、預測模型與即時決策，在作物健康方面將更能精準與精確地解決病蟲草害防治、植體營養與健康檢測，以及資源管理 (如土壤、水源、氣象、資材) 等挑戰，最終實現更有效

率、更佳質量生產、更永續的農業運作體系 (Agrawal & Arafat 2024)。在土壤分析與管理方面，配備農業人工智慧的農用無人機可以創建土壤特性的詳細地圖 (如 pH 值、水分含量及養分水準)，由農田土壤變化情況進行更有針對性與更有效的土壤管理 (如肥料投入、灌溉水量、微生物調理) 及土壤健康監測。必要時，農業人工智慧可以推薦土壤改良或作物輪作策略等措施來維持或提高土壤肥力，評估與確保土壤健康來促進永續利用，或調節灌溉、農藥及肥料的施用，以降低對環境與生態的負面影響。在生物多樣性方面，使用農業人工智慧優化病原菌與害蟲控制及營養管理，可以創造或改善野生動植物物種的生存環境，促進生物多樣性及提高生態系統的復原力，並有助於維護整體良好的農業景觀。

從瞭解適合各種農業任務的不同類型農用無人機到探索提供寶貴數據的複雜感測器技術，各個農業場域可以根據特定需求自訂或選購這些組件，而農業人工智慧與農用無人機的結合更深入地擴大了影響力，產生的協同效應可以使農業經營管理達到前所未有的精確水平。未來藉由簡便易行與高度自動化作業系統，使得所有技術背景的農民經過適度學習即可使用農用無人機，數據管理與分析軟體可以將複雜的數據集轉化為可操作的步驟。而特定應用的功能允許進行有針對性的干預/處理，從播種、病蟲草害防治到精確灌溉等，確保每一寸農田都得到最高效率的管理。

結語

農耕觀念的更新與科技的進步，使得農用無人機成為未來農業的空中 AI 助手，從單一作業機具進化為農業資訊/數據平台、逆境/災害偵 (監) 測耳目、防疫防災救援樞紐、農業淨零碳管理的多功能工具。臺灣農用無人機在農耕體系上的引用與利用，也從早期稻作栽培的噴藥施肥目的逐漸成為各類大面積栽植及經濟作物生產管理的高效率、低風險、數據化及多目標的解決方案。未來結合各種感測裝置、AI 分析及 IoT 整合等元素，農用無人機的廣泛應用可望進一步提升農糧生產的質量、農業經營管理的效能，貢獻於農業永續願景的實現。臺灣農村的高齡化與勞力短缺現象，提供了農用無人機未來更為寬廣應用領域及使用趨勢的契機。透過連結感測網絡、最新行動通訊技術、IoT、AI 大數據資料運算與分析、農耕作業機具及農用機器人等，農用無人機將可以串接智慧農業資訊整合平台 (智慧農業雲) 與尖端科技，打造更高效的精準智慧農耕體系，助益於更環保、更高利潤及更永續的農業發展。

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Agricultural Drones, a Modern Tool for Agricultural Multi-Applications and Future Perspectives

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Abstract

Chen, Y. J. and C. M. Yang. 2026. Agricultural drones, a modern tool for agricultural multi-applications and future perspectives. J. Taiwan Agric. Res. 75(1):73–85.

The renewal of farming concepts and the advancement of technologies have driven the improvement and innovation of agricultural management. From food production, agricultural product marketing, to agricultural environmental protection and ecological maintenance, the thinking and practices are progressing with the pulse of the times. It is hoped that human beings can live a happy life with enough food supply, and that people can live a bright and colorful life. Agricultural drones have emerged at this time, not only at the forefront of the current agricultural industry, but also becoming an aerial artificial intelligence helper for the future development of agriculture. They have evolved from a single operating tool to an agricultural information/data platform, an adversity/disaster detection (monitoring) eye and ear, an epidemic prevention and disaster prevention and rescue hub, and even a multi-functional tool for agricultural net zero and carbon management. The introduction and utilization of agricultural drones in Taiwan's farming system has gradually evolved from the early spraying and fertilizing of rice cultivation to a variety of applications. It is expected that, in the future, various sensing devices, advanced technologies, artificial intelligence, the Internet of Things, and customized operating systems can be combined to be widely used to further improve the quality of agricultural and food production, the efficiency of agricultural management, and a tool to cope with climate change and agricultural net zero. With that, it can provide data-based, multi-target, high-efficiency, low-risk, and multi-featured solutions to contribute to the realization of the vision of sustainable agriculture. This article integrates relevant information and scenarios to describe the advantages and challenges of agricultural drones. In view of the future, it hopes that agricultural practitioners will be far-sighted and insightful about opportunities, and agricultural stakeholders will find niches for industry development, and work together for mutual benefit and win-win results to usher in a new era of agriculture.

Key words: Drones, Agricultural drones, Agricultural applications, Precision agriculture, Smart agriculture.

Received: July 4, 2025; Accepted: November 27, 2025.

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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):87–101 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0005

Research Article

Symptomatology of *Fusarium oxysporum* Causing Pitaya Fusarium Fruit Rot in Taiwan

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Abstract

Fan, J. W., J. N. Tsai, and C. P. Lin. 2026. Symptomatology of the *Fusarium oxysporum* causing pitaya Fusarium fruit rot in Taiwan. J. Taiwan Agric. Res. 75(1):87–101.

Fusarium fruit rot is one of the common postharvest diseases of pitaya fruit in Taiwan. Since 2013, infected postharvest fruits were collected, exhibiting symptoms such as light brown patches with transverse stripes, white mycelium on the surface, or water-soaked lesions in severe cases. Green spots and less frequently observed scarring or rot on the pedicels and junctional branches of young fruits were also collected. All isolates obtained from these tissues exhibited morphological characteristics like those of *Fusarium oxysporum*. Further examination of colony morphology and sporulation patterns, combined with multilocus phylogenetic analyses based on RNA polymerase II second largest subunit (*rpb2*) and translation elongation factor 1-α (*tef1*) confirmed that all isolates belonged to *F. oxysporum*. Through artificial inoculation, symptoms on the postharvest fruits caused by *F. oxysporum* were confirmed, with symptoms showing a strong correlation with environmental humidity. When inoculation was performed on the surface of field-growing young fruits, green spots appeared after the color transition stage. Interestingly, consistent with the observations in the field, these spots did not progress to typical Fusarium fruit rot as the fruit matured, suggesting that the green spot and fruit rot symptoms may arise from infections occurring at different stages of fruit development. Also, the symptom of scarring and rot on pedicels were confirmed by the inoculation on the growing young fruits. This is the first disease report of the diversified symptoms on pitaya fruits caused by *F. oxysporum*, which can be attributed to different environment conditions and inoculation stage.

Key words: Pitaya, Postharvest disease, Fusarium fruit rot, *Fusarium oxysporum*.

INTRODUCTION

The most popularly cultivated pitaya fruit (*Hylocereus* spp.) in Taiwan can mainly be categorized by the flesh into white-fleshed type (*H. undatus* Britt. & Rose) and red-fleshed type (*H. polyrhizus* Britt. & Rose and *H. costaricensis* Britt. & Rose) (Jiang & Yang 2015). It is one of the important economic fruit trees in Taiwan. According to the latest statistical data, in 2024, the cultivation area was approximately 2,576.5 ha, with a fruit production value reaching 2.79 billion NTD (Agricultural Statistics Data Inquiry, Ministry of Agriculture, https://agrstat.moa.gov.tw/sdweb/public/inquiry/InquireAdvance.aspx).

Various *Fusarium* species have been widely reported as pathogens of pitaya in Malaysia, Indonesia, Argentina, and other regions. These include *F. fujikuroi* Nirenberg, *F. proliferatum* (Matsushima) Nirenberg, *F. solani* (Mart.)

Received: June 5, 2025; Accepted: October 31, 2025.
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Sacc., and *F. oxysporum* Schltdl., which cause symptoms such as stem rot and basal rot (Wright *et al*. 2007; Rita *et al*. 2013; Balendres & Bengoa 2019; Mohd *et al*. 2023). The study by Cui *et al*. (2011) identifies *F. oxysporum* to be the major pathogen responsible for postharvest fruit rot on imported pitaya in the market in China. In Taiwan, pitaya fruit diseases caused by *F. oxysporum* have been previously reported (Lin *et al*. 2014). Symptoms observed in diseased pitaya fruits included brown spots or postharvest rot with white mycelium. Moreover, *F. oxysporum* has also been isolated from mature fruits with green spots and from young fruits with rotted pedicels (Lin *et al*. 2023). However, previous reports have remained preliminary, without providing systematic scientific evidence to clarify the involvement of *F. oxysporum* in pitaya fruit rot or to comprehensively describe the diversity of its symptomatology.

In this study, diseased pitaya fruit samples were collected from various fields, and fungal isolates were obtained. The identities of these isolates were confirmed based on morphological and molecular characteristics. Furthermore, to comprehend the pathogenicity and symptom development of pathogens in both young and mature pitaya fruits, different inoculation conditions were tested to observe variations in symptoms.

MATERIALS AND METHODS

Fungal isolation and preservation

Survey locations included Waipu district, Taichung city; Erlin town, Changhua county; Mingjian township, and Jiji town, Nantou county; Erlun town, Yunlin county; Zhuqi township, Chiayi county; Dongshan district, Tainan county; Alian district, Kaohsiung city; Wandan township, Pingtung county; Sanxing township, Yilan county; and Yuli town, Hualien county.

Diseased fruits were collected and pathogen isolation were performed as followed: diseased tissues of approximately 4 × 4 × 1 mm^3 were excised, soaked in 5% NaClO for surface sterilization for 20–30 s, dried of excess surface droplets with paper towels, and then placed on potato dextrose agar (PDA, Difco^{TM}, Franklin Lakes, NJ, USA) plates and incubated at room temperature (RT, 22–26℃) for 2–3 d. To further purified the isolates, a single conidium of each isolates was selected and transferred to a new PDA plate, which were then incubated at RT under a 12-h light cycle for 7–10 d. For long-term preservation, agar blocks (approximately 5 × 5 × 2 mm^3) containing mycelia of isolates were excised and preserved in 100% sterile mineral oil (Sigma-Aldrich, Louis, MO, USA). Each isolate was maintained under its designated laboratory accession number, and detailed information is presented in Table 1.

Morphological observations

Representative isolates included F213214, obtained from fruit rot in Waipu District in 2013, and F220020, obtained in Alian District in 2020. These isolates were cultured on PDA plates and slants at 22–24℃ under continuous light for 7–10 d, and the colony morphology was observed. Microconidia were observed by culturing the isolates on PDA plates and slants under light at 22–24℃ for 3–7 d. Macroconidia were observed by culturing the isolates on PDA plates and slants for 3–14 d. Chlamydospores were observed by culturing the isolates on PDA slants for over 1 mo. For each spore type, the size of 50 randomly selected spores was measured. An Olympus BX40 microscope (Olympus, Tokyo, Japan) and Canon 50D camera (Canon, Tokyo, Japan) were used for the observations and photography.

Growth observations of isolates

The test isolates were cultured in the dark on PDA plates for 3–5 d. Fresh hyphal plugs were cut from the colony edges using a 0.6 cm agar punch and transferred to the edges of new PDA plates. To observe the optimum temperature for hypha growth of the isolates, these plates were incubated at temperatures ranging from 4 to 40℃ (in 4℃ intervals, totaling 10 temperatures). Each isolate was tested with 3 plates per temperature, providing 3 replicates. The growth rate of hyphae at different temperatures was measured. The linear growth distance was recorded daily. At

certain temperatures, the colonies covered the entire plate by day 10, so growth data from day 1–9 were measured. The daily growth rate for each plate was calculated as (growth distance at day 9 - growth distance at day 1) cm/8 d (unit: cm d^{-1}). The experiment was conducted 3 times, and the mean value was presented.

Molecular identification of pathogenic fungi

Extraction of fungal genomic DNA (gDNA)

For each isolate, mycelium was collected from PDA plates after incubation at 24℃ for 7–10 d. Genomic DNA was extracted using the Kaneka Easy DNA Extraction Kit version 2 (Funakoshi, Tokyo, Japan), and the extracted gDNA was stored at -20℃ for subsequent use.

Gene sequence amplification and sequencing

Genomic DNA extracted from the isolates was subjected to polymerase chain reaction (PCR) using specific primer pairs to amplify target sequences, including RNA polymerase II second largest subunit (*rpb2*) and translation elongation factor 1-α (*tef1*). The primer pairs used are listed in Table 2, and PCR conditions followed those described in the respective references with minor modifications.

After confirming the amplified products as single band via 1.5% agarose gel electrophoresis, they were sent to Tri-I Biotech Inc., Taiwan for

Table 1. List of the isolates which were isolated in this study and related information and accession numbers in GenBank.

Species	Isolates	Stages/symptoms	Year	Location	*rpb2*	*tef1*
F. oxysporum	F213124	postharvest/fruit rot	2013	Jiji	PP783793	PP731826
	F213214	postharvest/fruit rot	2013	Waipu	PP783794	PP731827
	F217077	young fruit/pedicel rot	2017	Erlin	PP783795	PP731828
	F217138	young fruit /green spot	2017	Erlin	PP783796	PP731829
	F217171	young fruit /green spot	2017	Erlun	PP783797	PP731830
	F218078	young fruit /pedicel rot	2018	Mingjian	PP783798	PP731831
	F218109	young fruit /pedicel rot	2018	Dongshan	PP783799	PP731832
	F219028	postharvest/fruit rot	2019	Waipu	PP783800	PP731833
	F213200	postharvest/fruit rot	2013	Zhuqi	PP783807	PP731840
	F219032	postharvest/fruit rot	2019	Alian	PP783808	PP731841
	F220020	postharvest/fruit rot	2020	Alian	PP783810	PP731843
	F221117	postharvest/fruit rot	2021	Alian	PP783811	PP731844
	F223034	postharvest/fruit rot	2023	Erlin	PP783812	PP731845
	F224057	young fruit/pedicel rot	2024	Mingjian	PV696124	PV696127
	F224071	young fruit/pedicel rot	2024	Erlin	PV696125	PV696128
	F224086	young fruit/pedicel rot	2024	Alian	PV696126	PV696129

Table 2. Primer pairs used for each gene region and polymerase chain reaction (PCR) conditions.

Gene/locus	Primer	Sequence (5'-3')	PCR amplification procedures	Reference
RNA polymerase II second largest subunit (*rpb2*)	5f2 7cr	GGGGWGAYCAGAAGAAGGC CCCATRGCTTGYTTRCCCAT	94℃: 90 s, (94℃: 30 s, 55℃: 90 s, 68℃: 2 min) × 40 cycles, 68℃: 5 min.	Liu *et al*. (1999); O'Donnell *et al*. (2007); Sung *et al*. (2007)
translation elongation factor 1-α (*tef1*)	EF1 EF2	ATGGGTAAGGARGACAAGAC GGARGTACCAGTSATCATGTT	95℃: 4 min, (94℃: 1 min, 55℃: 30s, 72℃: 90 s) × 35 cycles, 72℃: 10 min.	O'Donnell *et al*. (1998); Garmendia *et al*. (2018)

sequencing. If electrophoresis results showed multiple bands for the amplified products, the annealing temperature of PCR was increased to achieve a single, distinct amplification product before sequencing.

The raw sequences obtained from forward and reverse primers were assembled using Contig Express software (Vector NTI Advance 11.5, Invitrogen, Carlsbad, CA, USA), and unstable signal parts at both ends were trimmed to obtain the final sequencing result.

Phylogenetic analysis

The phylogenetic analysis was conducted using sequence data from 51 isolates, including 16 isolates in this study and 35 reference isolates obtained from the National Center for Biotechnology Information (NCBI) database. The reference isolates and outgroup were selected based on the closest matches obtained from Basic Local Alignment Search Tool (BLAST) searches using the sequences of the test isolates, and the general structure of *Fusarium* groups confirmed by published literature (Schroers *et al*. 2009; O'Donnell *et al*. 2012, 2013; Xia *et al*. 2019). Suitable isolate sequences were then retrieved from the NCBI database for comparison.

Sequences of the individual gene region were initially aligned using MAFFT Multiple Sequence Alignment Software Version 7 (Katoh & Standley 2013) with auto strategy. Gaps in both ends were deleted. These gene segments were subsequently used to construct two-gene (*rpb2* + *tef1*) concatenated phylogenetic trees. The phylogenetic tree was constructed using the maximum likelihood (ML) method. The optimal substitution model was predicted using MEGA X software (Kumar *et al*. 2018), with the model having the lowest Bayesian Information Criterion (BIC) value selected. The "use all sites" option was chosen for handling gaps and missing data.

The ML phylogenetic tree was constructed using MEGA X software, employing the heuristic Nearest-Neighbor-Interchange (NNI) method for tree searching. Branch support was tested with 1,000 bootstrap replicates.

Pathogenicity experiment

Test fruits of mature white-fleshed or red-fleshed pitaya were purchased from a farm in central Taiwan. Isolates F213214 and F220020 were chosen as test strains, cultured on PDA plates and incubated for 7–10 d under a 12-h photoperiod. Conidia were collected using sterile water, with microconidia comprising more than 95% of the spores. After filtering through sterilized gauze (8 layers), the spore concentration was adjusted to desired level for use as the inoculum. To fulfill Koch's postulates, mature fruits and field-growing fruits were independently inoculated, observed for the appearance of similar symptoms, and re-isolated to confirm the same pathogens.

Inoculation of mature fruits

Before inoculation, the fruits were surface-sterilized with 200 mg mL^{-1} NaClO for 1 min, then dried with paper towels. Each fruit was inoculated at 4 points, with or without wounded treatment. For wounding, a bundle of 10 insect pins (NO.3 DM0004-3; MegaView) was used to gently prick the fruit surface 15–20 times to a depth of about 1 mm. Sterile cotton, approximately 1 cm^3 in volume, was soaked in the spore suspension (1–5 × 10^6 spores mL^{-1}), stirred, and left to stand for over 3 min. During inoculation, the soaked cotton was picked up with tweezers, excess liquid was removed, and the cotton was placed on each inoculation point, secured with transparent tape. The inoculated fruits were then placed in a preservation box with a paper towel moistened with sterile water at the bottom to maintain relative humidity (RH) > 95%. The box was sealed and stored in a dark room at RT (24–28℃).

For the high and low humidity symptom comparison experiment, 10 μL of the spore suspension was dropped onto the inoculation point, followed by 10 μL of Bacto agar (Difco™, Franklin Lakes, NJ, USA). High humidity (avg. RH = 97.7% ± 3.3%) was maintained by sealing the preservation box lid, while low humidity (avg. RH = 78.7% ± 8.3%) was achieved by

partially covering the box. Temperature and humidity within the boxes were monitored using a temperature and RH data logger (MX2301, HOBO, Onset Computer Corporation, Bourne, MA, USA). The control group was treated with sterile water instead of the spore suspension. The experiment was conducted 3 times and showed similar results.

Inoculation of field-growing fruits

Beside the representative isolates, 2 additional isolates, F217077 and F218109, were included in the experiment. From June to September 2021, fruits at the initial fruiting stage (0 wk) from white-fleshed pitaya fruit plants grown at the field of Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC were selected for inoculation. The fruit surface or pedicel was pricked 10–15 times with insect pins. Sterile cotton soaked in a 2–3 × 10^6 mL^{-1} spore suspension was then squeezed with tweezers and fixed to the wound with transparent tape. The fruits were covered with kraft paper bags to minimize rainwater entry. Inoculations were performed weekly from the 0-wk stage until the fruits were about 2 wk old, just before color transition stage, with a total of 3 inoculations. New cotton was used for each inoculation. One wk after the third inoculation, the cotton was removed, and the fruits were rewrapped in kraft paper bags until maturity. The experiment was conducted 3 times and showed similar results.

After harvesting, the fruits were placed in plastic baskets with non-slip mats and paper towels at the bottom, with the addition of sterile water. The baskets were sealed with plastic wrap, with holes poked in the 4 corners for ventilation, and kept at RT for 1 wk for observation.

RESULTS

Symptom description of *F. oxysporum* on pitaya fruits

From 2013 to 2024, postharvest pitaya fruits exhibiting various disease symptoms were continuously collected across multiple regions in Taiwan. Observed symptoms on mature fruits of both white- and red-fleshed pitaya included light to dark brown spots with fine transverse stripes or white mycelium growth (Fig. 1A and B). In severe cases, water-soaked symptoms were also observed (Fig. 1B). Fungi showing morphological traits typical of *F. oxysporum* were repeatedly isolated from these symptomatic tissues. Notably, fungi with similar characteristics were also isolated from green spots on the fruit surfaces (Fig. 1C), with scabbed pedicels of young fruits, and rotting on the junctions of the branches (Fig. 1D–F). These symptomatic cases are widespread and commonly observed in commercial orchards throughout Taiwan. To distinguish it from rot caused by other pathogens, the fruit rot specifically caused by *Fusarium* species is referred to as "Fusarium fruit rot".

Morphological characteristics of *F. oxysporum* isolates

The morphology of representative isolates, F213214 and F220020, were examined. Isolates F213214 exhibits the following features: the front of the colony is white to pale vinaceous, while the reverse side is deeper reddish-purple (Fig. 2A and B). The colony edges are filiform with abundant and fluffy aerial mycelium. Microconidia are hyaline, ellipsoidal, with blunt ends, occasionally with 1 end slightly pointed, with 0–1 septa, measuring 4.0-(7.3)-11.0 μm (min.-avg.-max.) in length and 2.1-(2.5)-4.2 μm in width, with a length-to-width ratio of 1.3-(2.9)-5.5 (Table 3, Fig. 2C). Macroconidia are hyaline, long stick-shaped, with 2–4 septa, apical cells blunt to papillate, and basal cells foot-shaped, measuring 24.3-(43.0)-72.0 μm in length and 2.8-(4.5)-6.1 μm in width, resulting in a length-to-width ratio of 5.8-(9.6)-16.1 (Table 3, Fig. 2D). Chlamydospores are globose to subglobose, with a diam. of 5.4-(7.2)-8.9 μm (Table 3, Fig. 2E). The optimal growth temperature for the mycelium is 28℃, with growth nearly ceasing at 4℃ and no growth above 40℃. Isolate F220020 of *F. oxysporum* exhibited morphological characteristics similar to those of isolate F213214 (Fig. 2F–J). However, the macroconidia exhibited the typical hook-

shaped apical cells with 3–4 septa (Fig. 2I). The morphology of both representative isolates closely resembles the description of *F. oxysporum* in the literature (Leslie & Summerell 2006).

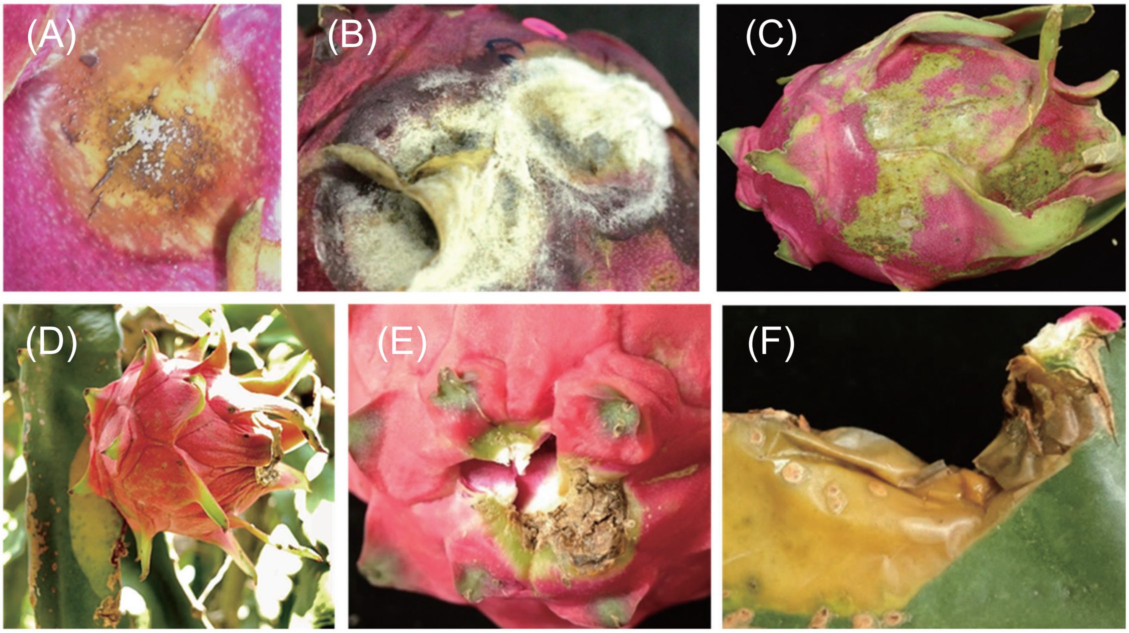

Fig. 1. The symptoms of pitaya Fusarium rot caused by *Fusarium oxysporum* in field, including (A, B) brown spots with transverse stripes or white mycelium on mature fruits, (C) green spots, and (D, E) scabbed pedicels of young fruits and (F) rotting on the junctional branch.

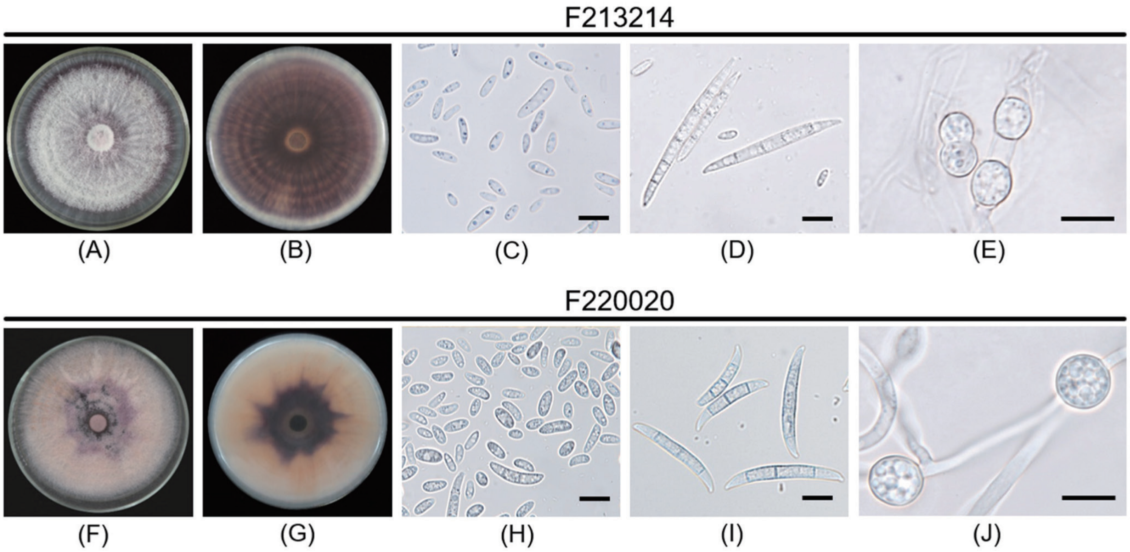

Fig. 2. Morphological features of the *Fusarium oxysporum* isolates F213214 and F220020. (A, F) Top and (B, G) reverse views of a colony. (C, H) Microconidia, (D, I) macroconidia, and (E, J) chlamydospore of each isolate were illustrated in one row from left to right. Bar = 10 μm.

Phylogenetic analysis of the *F. oxysporum* isolates

A ML phylogenetic tree was constructed using concatenated sequences of *rpb2* and *tef1* genes (Fig. 3). The analysis included 16 isolates from this study (Table 1), 30 *Fusarium* reference isolates, and 1 outgroup (*Cylindrocarpon cylindroides*), all obtained from published literature (Table 4). All 16 isolates in this study belonged to the *F. oxysporum* clade, supported by a 98% bootstrap value.

Both morphological and molecular identification results confirm that the two representative isolates belong to *F. oxysporum*.

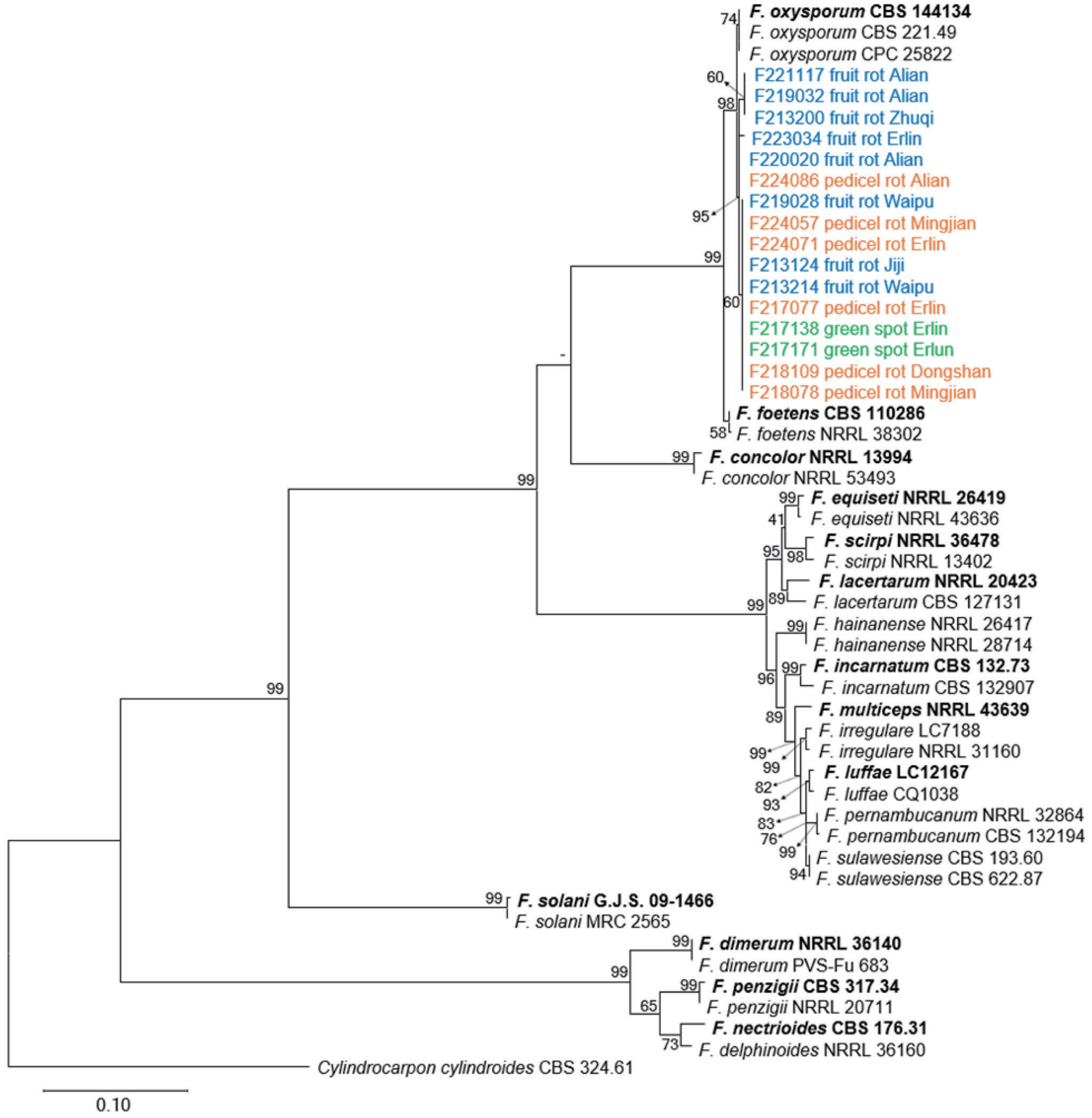

Fig. 3. Maximum likelihood (ML) phylogenetic tree based on RNA polymerase II second largest subunit (*rpb2*) and translation elongation factor 1-α (*tef1*) sequences of *Fusarium* spp. Isolates from this study are color-coded based on associated symptoms: blue for fruit rot, green for green spot, and orange for pedicel rot. Type strains are displayed in bold. Bootstrap support values (≥ 50%) are displayed at the nodes. The tree was rooted to *Cylindrocarpon cylindroides* CBS 324.61.

Table 3. Synopsis of morphological and physical data of the *Fusarium oxysporum* isolates in Taiwan.

Isolate no.	Microconidi length × width min.-avg.-max (μm), Length width ratio	Macroconidia length × width min.-avg.-max (μm), Length width ratio	Chlamydospore diameter min.-avg.-max (μm)	Optimum temperature for mycelium growth	Mycelium growth rate at optimum temperature (avg. ± *SE*, mm d^{-1})
F213214	4.0-(7.3)-11.0 × 2.1-(2.5)-4.2, 1.3-(2.9)-5.5	24.3-(43.0)-72.0 × 2.8-(4.5)-6.1, 5.8-(9.6)-16.1	5.4-(7.2)-8.9	28℃	5.6 ± 0.1
F220020	4.1-(6.6)-12.0 × 2.2-(2.8)-4.1, 1.3-(2.5)-4.5	24.1-(34.6)-49.1 × 3.2-(4.2)-6.0, 5.6-(8.4)-13.7	5.3-(8.9)-12.2	28℃	6.0 ± 0.0

Table 4. List of the reference isolates used in this study and related information and accession numbers.

Species	Isolates	*rpb2*	*tef1*	Reference
F. concolor	NRRL 13994[T] = CBS 183.34	MH742569	MH742650	Crous *et al*. (2022)
	NRRL 53493	MH742614	MH742685	Jacobs-Venter *et al*. (2018)
F. delphinoides	NRRL 36160	HM347219	HM347134	O'Donnell *et al*. (2010)
F. dimerum	NRRL 36140[T]	HM347218	HM347133	O'Donnell *et al*. (2010)
	PVS-Fu 683	MN540014	MT742267	Balmas *et al*. (2021)
F. equiseti	NRRL 26419[T]	GQ505777	GQ505599	O'Donnell *et al*. (2009b)
	NRRL 43636	GQ505841	GQ505663	O'Donnell *et al*. (2009b)
F. foetens	CBS 110286[T]	MT010984	MT011001	Brankovics *et al*. (2020)
	NRRL 38302	JX171652	FJ985444	O'Donnell *et al*. (2009a, 2013)
F. hainanense	NRRL 26417	GQ505776	GQ505598	O'Donnell *et al*. (2009b)
	NRRL 28714	GQ505782	GQ505604	O'Donnell *et al*. (2009b)
F. incarnatum	CBS 132.73[T]	MN170409	MN170476	Xia *et al*. (2019)
	CBS 132907	MN170410	MN170477	Xia *et al*. (2019)
F. irregulare	LC7188	MK289783	MK289629	Wang *et al*. (2019)
	NRRL 31160	GQ505785	GQ505607	O'Donnell *et al*. (2009b)
F. lacertarum	CBS 127131	MN120739	MN120758	Lombard *et al*. (2019b)
	NRRL 20423[T]	JX171581	GQ505593	Lombard *et al*. (2019b)
F. luffae	CQ1038	MK289723	MK289569	Wang *et al*. (2019)
	LC12167[T]	MK289754	MK289601	Wang *et al*. (2019)
F. multiceps	NRRL 43639[T]	GQ505844	GQ505666	O'Donnell *et al*. (2009b)
F. nectrioides	CBS 176.31[T]	JX171591	EU926312	Schroers *et al*. (2009) O'Donnell *et al*. (2013)
F. oxysporum	CBS 144134[T]	MH484953	MH485044	Lombard *et al*. (2019a)
	CBS 221.49	MH484872	MH484963	Lombard *et al*. (2019a)
	CPC 25822	MH484943	MH485034	Lombard *et al*. (2019a)
F. penzigii	CBS 317.34[T]	KM232362	EU926324	Lombard *et al*. (2015)
	NRRL 20711	HM347217	HM347132	O'Donnell *et al*. (2010)
F. pernambucanum	CBS 132194	MN170425	MN170492	Xia *et al*. (2019)
	NRRL 32864	GQ505791	GQ505613	O'Donnell *et al*. (2009b)
F. scirpi	NRRL 13402	JX171566	GQ505592	Sandoval-Denis *et al*. (2018)
	NRRL 36478[T]	GQ505832	GQ505654	O'Donnell *et al*. (2009b)

Table 4. List of the reference isolates used in this study and related information and accession numbers. (continued)

Species	Isolates	*rpb2*	*tef1*	Reference
F. solani	G.J.S. 09-1466[T]	KT313623	KT313611	Schroers *et al.* (2016)
	MRC 2565	MH582410	MH582420	O'Donnell *et al.* (2018)
F. sulawesiense	CBS 193.60	MN170435	MN170502	Xia *et al.* (2019)
	CBS 622.87	MN170436	MN170503	Xia *et al.* (2019)
outgroup *Cylindrocarpon cylindroides*	CBS 324.61	DQ789803	JF735788	Castlebury *et al.* (2006)

[T] Type strains.

Pathogenicity of *F. oxysporum* on mature fruits

Pathogenicity experiments were conducted on mature white-fleshed pitaya fruits. The results showed similar symptoms for both representative isolates. Under high humidity, brown water-soaked lesions appeared at the inoculation points 3–5 d after inoculation at RT and RH > 95%, accompanied by white fungal colony growth, and the same pathogens were re-isolated (Fig. 4A, B). Similar results were observed when the same isolates were tested on the red-fleshed pitaya fruits (Fig. 4C and D). Symptoms developed in both wounded and unwounded treatments, but progressed more rapidly in the wounded fruits (data not shown).

To confirm the effect of humidity on symptom development, inoculated fruits were placed under relatively low RH instead of high RH conditions. Under relatively low RH, both representative isolates caused similar symptoms: light brown spots, without water-soaking, appeared at the inoculation points 3–5 d after inoculation (Fig. 4E and F).

These symptoms described above resembled those commonly observed under field conditions. The same pathogens could be re-isolated from the symptomatic tissues, thereby fulfilling Koch's postulates. In the control group, some inoculation sites developed brown scabs indicative of physiological wounds (data not shown), which were distinct from symptoms caused by *F. oxysporum*, and no suspected pathogens were re-isolated from these sites.

Green spots and pedicel rot caused by *F. oxysporum*

To confirm the relationship between green spot symptoms and *F. oxysporum*, spore suspensions of the F213214 and F220020 isolates were individually inoculated on the surface of 0-wk-old white-fleshed pitaya fruits in the field. The symptoms were monitored continuously until the fruits changed color, matured, and then were harvested. After the fruits changed color, green spot symptoms appeared at the inoculation points with both isolates (Fig. 5A and B). However, after mature fruit harvesting, when the inoculated fruits were placed in a RT and high humidity environment, the green spots did not expand, nor did they develop into typical symptoms such as blocky brown spots or water-soaked rot caused by *F. oxysporum*. So did the natural infected samples with green spots.

To confirm the symptoms of scabbed pedicels and rotting on junctional branches caused by *F. oxysporum*, both representative isolates were tested by inoculating the pedicels of young fruits of white-fleshed pitaya. Inoculation with the *F. oxysporum* representative isolate F213214 resulted only in green spots (Fig. 5C). Additional isolates of *F. oxysporum*, F217077 and F218109, exhibited similar responses as the F213214 isolate (data not shown). However, inoculation with the other representative isolate F220020 caused infection symptoms at the junctions of pedicels and branches (Fig. 5D and E), similar to those observed in field pedicel tissues.

The same pathogens were re-isolated from all the symptomatic tissues, confirming their

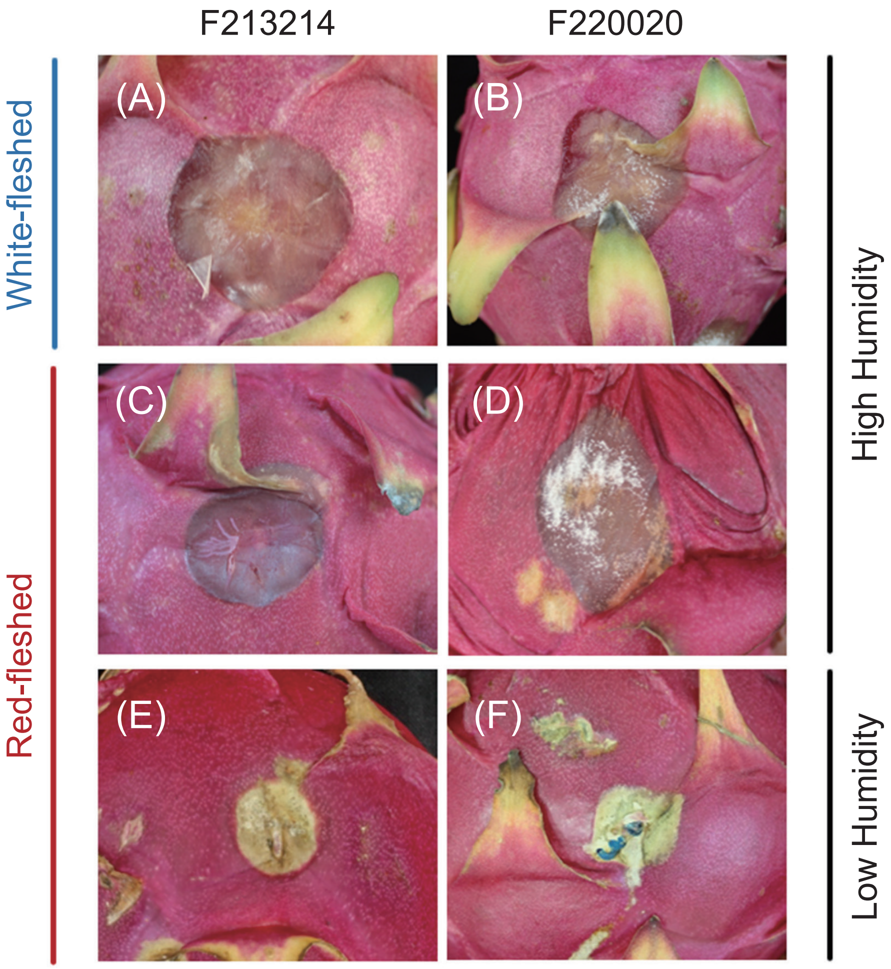

Fig. 4. Symptoms of artificial inoculation mature pitaya fruits, with test strains F213214 and F220020 (*Fusarium oxysporum*). (A, B) on white-fleshed fruits; (C–F) on red-fleshed fruits.

pathogenicity and thus, fulfilling the Koch's postulates. The control group showed no significant symptoms, and no suspected pathogens were isolated.

DISCUSSION

Based on the morphological, sequence analyses, and pathogenicity test, it is confirmed that *F. oxysporum* can induce a diverse range of symptoms on pitaya (Fig. 1). Postharvest fruits exhibit both dry brown spot symptoms and water-soaked lesions, with a strong correlation to humidity (Fig. 4); whereas young fruits may develop green spots, pedicel scabs, and rot at the junctional branches.

Intriguingly, through artificial inoculation experiments, it was revealed that postharvest symptoms only appeared in experiments where mature fruits were directly inoculated with *F. oxysporum*. No fruit rot symptoms occurred after harvest when inoculated on immature fruits before color transition stage (Fig. 5). From this, it can be deduced that the timing of infection for Fusarium fruit rot, which occurs after harvest, is close to maturity of fruits.

The same fungal isolates were re-isolated from the green spot symptoms, indicating the

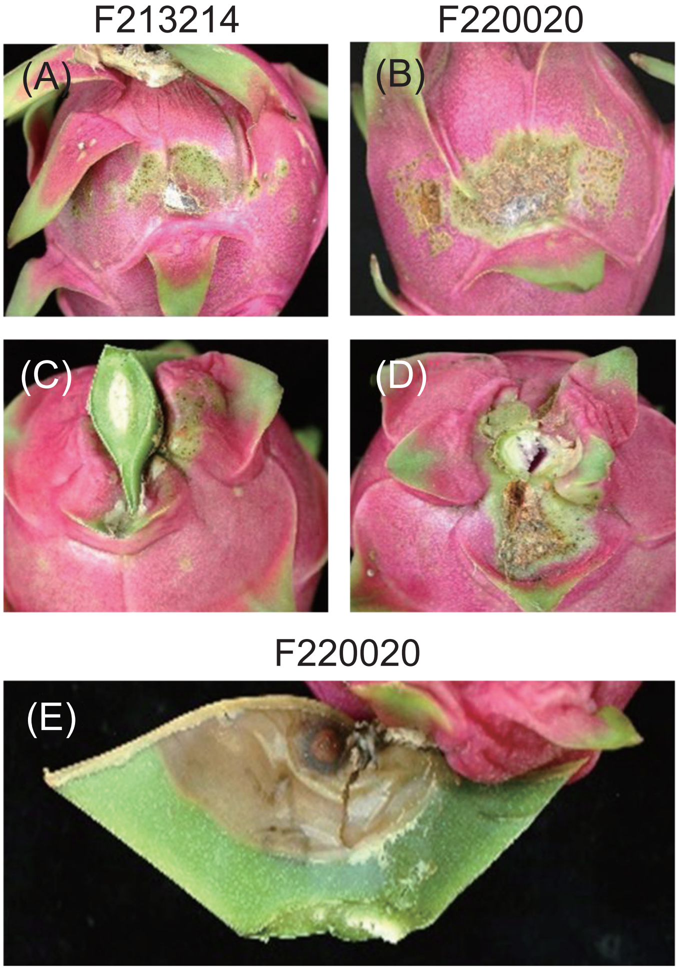

Fig. 5. Symptoms of artificial inoculation on young pitaya fruits in the fields, with strains F213214 and F220020 (*Fusarium oxysporum*). (A, B) inoculated on fruit surface; (C–E) inoculated at the junction of pedicel and branch.

persistence of the pathogen at the inoculation sites even after fruit maturation. Pitaya fruits are green during their immature stage, gradually turning red during maturation due to factors such as chlorophyll degradation and anthocyanin accumulation during the late developmental stage (Wybraniec *et al*. 2007; Phebe *et al*. 2009). Additionally, young fruits contain higher levels of phenolic compounds compared to mature fruits (Singh *et al*. 2022). Phenolic compounds are not only related to plant physiology but are also highly correlated with the ability to resist pathogen invasion (Pratyusha 2022). It is speculated that when young fruits are infected by *F. oxysporum*, they trigger specific defense mechanisms such as maintaining higher levels of phenolic compounds (Kumar *et al*. 2020) or other secondary metabolites with antimicrobial properties (Zaynab *et al*. 2018), accumulating high amounts of callose (Luna *et al*. 2011), altering tissue structure, or producing papillae to form barriers (Underwood 2012). These responses may confine the pathogen to the infection sites, preventing further fruit rot, but causing abnormal pigment metabolism that results in green spot formation.

In field surveys, *F. oxysporum* was found to cause pedicel scabs or rot at the junctional

branches occasionally. In artificial inoculation experiments, only 1 of the 4 isolates used in the tests could cause similar symptoms and fulfill the Koch's postulates. The conditions for pedicel and branch rot caused by *F. oxysporum* in the field require further study.

Comprehensive field investigations are needed to clarify these findings and to better understand the ecological dynamics of *F. oxysporum* in pitaya cultivation. The potential sources of infection remain uncertain. Although *F. oxysporum* is typically regarded as a soilborne pathogen (Joshi 2018; Srinivas *et al*. 2019), the occurrence of symptoms on aerial fruit tissues in this study suggests alternative modes of dissemination. One possibility is that wind and rain splash soilborne propagules onto the fruits; another is that small insects or snails may contribute to pathogen dispersal. Further studies are warranted to test these hypotheses. Interestingly, there have been report of stem rot in pitaya caused by *F. oxysporum* infection in Taiwan (Tsai 2004). However, *F. oxysporum* exhibits host-specific specialization through its *formae speciales*, and the pathogenicity among different isolates or *formae speciales* is highly variable. Moreover, several studies have considered *F. oxysporum* as a species complex (Laurence *et al*. 2014; Brankovics *et al*. 2020; Panno *et al*. 2021). Further studies are required to determine whether *F. oxysporum* isolates responsible for stem rot can also cause symptoms in aerial parts of pitaya, such as pedicel scabs, branch rot at the junctions, and fruit rot.

In conclusion, this study systematically confirms that *F. oxysporum* is the primary causal agent of pitaya fruit rot in Taiwan and is responsible for a wide spectrum of symptoms. The variation in symptom expression may be influenced by environmental humidity and the timing of infection. However, the conditions under which *F. oxysporum* causes pedicel rot remain to be studied. Additionally, the ecology of *F. oxysporum* needs to be further investigated to efficiently prevent the occurrence of Fusarium fruit rot.

ACKNOWLEDGMENTS

This article was supported by funding from the Taiwan Agricultural Research Institute, Ministry of Agriculture under projects 112 Agricultural Science-5.3.1-Agriculture-C1(4) and 113 Agricultural Science-5.3.1-Agriculture-01(3). Assistant Er-Xuan, Li aided in the investigation and experimentation process.

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引起臺灣紅龍果鐮孢果腐病 *Fusarium oxysporum* 之病徵探討

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摘要

范浚維、蔡志濃、林筑蘋。2026。引起臺灣紅龍果鐮孢果腐病 *Fusarium oxysporum* 之病徵探討。台灣農業研究 75(1):87–101。

鐮孢果腐病 (Fusarium fruit rot) 為臺灣紅龍果常見的採收後果實病害之一。自 2013 年起，採集罹染鐮孢果腐病的採收後果實，包含果表帶有橫紋或白色菌絲之塊狀褐斑，嚴重者呈水浸狀腐爛；或田間果實表面綠斑、幼果果梗處結痂及連接處腐爛等病徵。病徵經過組織分離後皆可得與 *Fusarium oxysporum* 形態特徵相似的分離株。進一步觀察菌落與產孢形態，以及利用 RNA polymerase II second largest subunit (*rpb2*) 與 translation elongation factor 1-α (*tef1*) 等 2 序列片段做多重序列親緣關係分析，確認皆屬 *F. oxysporum*。透過人工接種採收後果實，確認 *F. oxysporum* 引起之採後果實病徵，且病徵表現與環境濕度關係性高；接種於田間幼果表面時，接種點於果實轉色期後呈現綠斑，有趣的是，與田間發現的綠斑果實狀況一致，後續果實成熟後不會進展為鐮孢果腐病。亦透過接種田間幼果果梗確認 *F. oxysporum* 菌株可引起結痂或腐爛。本研究為首篇確認 *F. oxysporum* 可引起紅龍果果實多型病徵之報導，並確認其病徵差異與環境條件以及接種時機有關。

關鍵詞：紅龍果、採後病害、鐮孢果腐病、*Fusarium oxysporum*。

投稿日期：2025 年 6 月 5 日；接受日期：2025 年 10 月 31 日。
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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):103–116 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0006

研究報告

次氯酸鹽溶液防治紅龍果採後濕腐病之效果評估

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摘要

林筑蘋、黃巧雯、黃晉興、蔡志濃、徐敏記。2026。次氯酸鹽溶液防治紅龍果採後濕腐病之效果評估。台灣農業研究 75(1):103–116。

紅龍果 (*Hylocereus* spp.) 為具重要經濟價值的作物之一，而由 *Gilbertella persicaria* 於田間與採後引起的濕腐病為紅龍果產業的重大問題。本研究旨在探討次氯酸鹽溶液 (pH 8.1–8.4) 對紅龍果果實採後濕腐病的防治效果。在培養基上抑制 *G. persicaria* 的試驗中，在菌絲方面，有效氯濃度到 200 mg L^{-1} 的次氯酸鹽溶液仍未顯示出良好的抑制效果；而在孢子發芽方面，有效氯濃度達 150 mg L^{-1} 以上時，即可有效抑制孢子發芽。進一步以人工接種 *G. persicaria* 的方式，並以白肉種紅龍果果實為供試材料，結果顯示，於採後處理有效氯 200 mg L^{-1} 的次氯酸鹽溶液處理 30–60 秒，可顯著降低濕腐病罹病級數 (依照發病面積低到高給予 0–4 級數)。針對處理時機的結果顯示，在接種後 12 小時內施用上開處理都可以顯著降低濕腐病罹病級數，但若超過 24 小時才處理，其罹病級數則與對照組無顯著差異；然而同樣的處理條件對紅肉種紅龍果人工接種濕腐病菌則無防治效果。此外，測試供試藥劑對田間自然感染果實的採收後處理效果，顯示施用有效氯濃度 200 mg L^{-1} 的次氯酸鹽溶液條件後，白肉種果實的濕腐病平均發病率由未施用的 43.0 ± 6.7% 降為 9.4 ± 4.5%，防治率達 79.0 ± 11.3%；然而，在紅肉種果實上反而造成更嚴重濕腐病發病率，平均發病率由未施用的 43.0 ± 20.8% 提升為 69.2 ± 13.9%。本研究使用的次氯酸溶液處理有助於降低白肉種紅龍果的濕腐病，而紅肉種紅龍果的採後處理條件則尚需另外評估。

關鍵詞：*Gilbertella persicaria*、採後處理、最適濃度與時間、處理時機。

前言

臺灣種植的紅龍果 (pitaya, dragon fruit) 大致可分為白肉種與紅肉種，其中白肉種又稱『紅皮白肉種』(*Hylocereus undatus* Britt. & Rose)；紅肉種則分為『紅皮紅肉種』(*H. polyrhizus* (Weber) Britt. & Rose) 與『紅皮紫肉紅龍果』(*H. costaricensis* (Weber) Britt. & Rose) (Huang & Lin 2009)。但因為多年來引進的種類繁多，加上民間自行育種，以致紅肉品系繁雜、紫肉種及紅肉種難以區分 (Chang 2006)，因此紅肉種果實或統稱為『改良紅肉種』(*H. polyrhizus*; *H. costaricensis*) (Liu & Yu 2021)，本文後續簡稱為『紅肉種』。

由 *Gilbertella persicaria* (E. D. Eddy) Hesselt 引起的紅龍果病害，可造成田間莖部、花器、幼果及成熟果採後病害，在臺灣、中國大陸、泰國、墨西哥、日本及越南等地引起嚴重的損失 (Taba *et al*. 2011; Guo *et al*. 2012; Lin *et al*. 2014; Carrillo-Fasio *et al*. 2022; Vo *et al*. 2024)。在臺灣，已知 *G. persicaria* 可造成田間花器、幼果，以及採收後成熟果實腐爛，稱為『紅龍果濕腐病』(Lin *et al*. 2014)。其中 *G. persicaria* 偏好於高溫高濕生長 (Lin

投稿日期：2025 年 9 月 5 日；接受日期：2025 年 10 月 30 日。
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et al. 2014; Taba *et al*. 2022)，受感染的成熟果實若環境不適合不會發病，外觀看似正常；待貯藏或經過常溫宅配等高溫高濕的環境下才會發病，且發病進展快，數日內即可造成全顆果實腐爛，為常溫下紅龍果採收後最棘手的病害之一。為與田間花期濕腐病作區別，特將此稱為『紅龍果採後濕腐病』(Lin *et al*. 2020)。

防治紅龍果採後濕腐病的文獻有限，Vo *et al*. (2024) 探討根圈細菌防治 *G. persicaria* 的可能性；Li *et al*. (2019) 亦發現紅龍果處理以 β-胺基丁酸 (β-aminobutyric acid) 可降低 *G. persicaria* 對果實的危害，然而防治效果僅限在培養基或實驗室階段。果實消毒是指針對果實上的微生物達到消除活性的目的。在過去數十年來國內外均有對新鮮蔬果進行採後處理的相關消毒方式 (Pao *et al*. 2007; Zhou *et al*. 2021)。次氯酸鹽類已被證實對多種植物細菌、卵菌及病毒具有殺菌效果，殺菌成分包括次氯酸 (hypochlorous acid; HOCl) 與次氯酸鹽 (hypochlorite; OCl^-)，具強氧化能力可破壞微生物的細胞壁、細胞膜或外殼，進而達到抗菌功效，且取得方便，早已被應用在農業供採收後處理 (Copes & Ojiambo 2021; Boecker *et al*. 2023)。目前次氯酸鹽類用於紅龍果的病害防治效益尚未有相關報告，但在萵苣、木瓜、蘋果、藍莓、草莓及哈密瓜等蔬果上的試驗可知，施用氯系藥劑有助降低櫥架期間病原菌或其他微生物含量，進而延長櫥架壽命 (Shin *et al*. 2012; Chiabrando *et al*. 2017; Copes & Ojiambo 2021; Getnet *et al*. 2023)。美國亦曾建議保持洗滌槽與水槽 3,000 與 6,000 mg L^{-1} 之氯 (chlorine) 濃度以各別控制番茄與柑橘腐敗 (Suslow 2000)。

本研究探討次氯酸鹽溶液對抑制 *G. persicaria* 生長的效果，以及測試次氯酸鹽防治紅龍果果實採後濕腐病之最適施用條件，並且討論次氯酸鹽溶液防治效果在白肉與紅肉種間的差異性及使用的限制性；進一步自田間或合作社蒐集外觀正常的果實，測試次氯酸鹽溶液應用於田間自然感染果實時的防治狀況。

材料與方法

試驗菌株與供試果實製備

本研究使用的 *G. persicaria* 菌株 F218061、F218072 以及 F216139 皆分離自罹染濕腐病之紅龍果 (*Hylocereus* sp.) 花或果實。罹病組織經 0.5% NaClO 表面消毒 30 秒，以擦手紙吸乾表面多餘水分後，放置於馬鈴薯葡萄糖瓊脂 (potato dextrose agar; PDA, BD Difco™, Franklin Lakes, NJ, USA) 平板培養基上，於室溫 (24–28℃) 下放置 1–2 日，切取自罹病組織長出之單一菌絲尖端，移植於新的 PDA 中培養，靜置於室溫下經黑暗培養 3 日，切取 $10 \times 10 \times 0.5$ mm^3 菌塊，移植於無菌礦物油 (100% mineral oil, Sigma-Aldrich, St. Louis, MO, USA) 中保存。本研究中用來測試果實適用之次氯酸鹽條件的白肉種 (*H. undatus*) 與紅肉種「大紅」(*Hylocereus* sp. cv ‘Da-Hong’)，購自臺中市外埔區與彰化縣二林鎮果園所產之果實。果實試驗前以自來水清洗果表，以 75% 酒精噴布整顆果實後，用紙巾擦乾。

次氯酸來源與濃度測定

次氯酸鹽溶液製作方式為以 50 g 氯化鈉 (臺鹽高級精鹽-未加碘；臺鹽實業，臺灣苗栗縣) 溶解於 5 L 之無菌水中，以次氯酸電解水製造系統 (型號 YH520G；奕泓環境科技有限公司，臺灣臺中市) 經電解產生含次氯酸、次氯酸根離子及氫氧化鈉的混合溶液。之後以酸鹼度測定儀 (Denver Instrument, Inc., Arvad, CO, USA) 測量 pH 值，並以 “eXact® Micro7+” (Industrial Test Systems, Inc., Rock Hill, SC, USA) 配合試劑 “eXact® Strip Micro HR” (試劑編號 486672；Industrial Test Systems, Inc., Rock Hill, SC, USA) 測定有效氯濃度，並按照試驗需求於當日配置所需的濃度。配製好的溶液存放於避光、密閉的容器內，從確認濃度到使用完畢不超過 24 小時。經測試，有效氯濃度在至少 24 小時內皆維持穩定，未出現顯著降低的狀況。

次氯酸鹽在培養基上對菌絲與孢子的抑制效果

抑制孢子發芽測試

供試菌株以避光方式培養於馬鈴薯葡萄糖瓊脂培養基上 7 日 (28℃)，以塑膠滴管沾取表面之孢子堆，並混入裝有不同濃度次氯酸鹽的燒杯中。孢子與次氯酸混合液混合 1 分鐘後吸取 10 µL 至 PDA 培養基上，12 滴/皿。對照組

為孢子與無菌水混合。在避光與 28℃培養 16 小時後以光學顯微鏡計算孢子發芽數量。每次每菌株每組分別計數 500 個孢子計算出發芽率，試驗共重複 3 次。

菌絲生長抑制測試

以0.5 mm打孔器切取供試菌株菌絲邊緣，將菌絲塊挑起個別放入 0、50、100、150 或 200 mg L^{-1} 等次氯酸鹽溶液浸泡 1 分鐘後，將菌絲塊挑起，放置於紙巾上吸乾多餘水分，將菌絲面朝下平貼於 PDA 中央，28℃培養 24 小時後進行測量。對照組為菌絲塊放入無菌水中。每菌株每處理進行 4 皿得每次平均值，試驗共進行 3 次重複。

次氯酸鹽溶液處理果實最適條件試驗

病原菌接種

以 *G. persicaria* F218061 分離株為供試菌株。孢子懸浮液製備方式與接種方式如 Lin *et al*. (2014) 所示，惟修改為以 5×10^4 spores mL^{-1} 孢子懸浮液噴灑接種果實，直到每粒供試果實有水滴滑落後，再放置於室溫等待下一步處理。

最適使用濃度與時間

待測果實為白肉種紅龍果。於找出最適浸泡濃度的試驗中，果實於接種病原菌的 0.5 小時後 (hour-post inoculation; hpi)，各別浸泡 50、100、150、200 mg L^{-1} 等不同有效氯濃度之次氯酸鹽溶液 1 分鐘，每組 4–8 粒果實 (biologicalreplicates，生物重複數)，試驗重複 5 次 (experimental repeats)；於最適濃度與其浸泡時間的試驗中，果實則是於 0.5 hpi 各別浸泡 100 mg L^{-1} 之次氯酸鹽溶液 1、5 或 10 分鐘、200 mg L^{-1} 之次氯酸鹽溶液 1、5 或 10 分鐘，每處理 5–7 粒果實，試驗重複 4 次。對照組為接種後不經過浸泡次氯酸鹽溶液的『未處理組 (untreated)』，以及無菌水取代次氯酸溶液的『水處理組 (water)』，浸泡時間固定為 1 分鐘。浸泡時輕攪動果實使果實在溶液中輕輕滾動。經浸泡過次氯酸溶液後，直接將果實放置於半密閉容器內，於 24–30℃、濕度 RH > 95%，以及避光的環境，4–5 日後觀察濕腐病發病狀況 (Lin *et al*. 2014)。

於紅肉種果實測試時，接種條件同上，但以紅肉「大紅」品種為供試植物，並於 0.5–3 hpi 處理以次氯酸鹽 30 秒。對照組為接種後不再經過處理的『未處理組』，以及無菌水取代次氯酸溶液的『水處理組』。試驗重複 3 次，每組 7 粒果實。

最適使用時機

以白肉種果實供試，每一次皆是以同一批果實均分成 9 組 (未處理組、處理組及水對照組各 4 個感染後時間點)，每組 7 粒果實，並且同時接種，各自於 0.5、6、12、24 hpi，以有效氯 200 mg L^{-1} 的次氯酸鹽溶液 (pH 8.1–8.4) 或水，放置於 24–30℃、保持濕度 RH > 95% 且避光的環境，統一於接種 4–5 日後觀察濕腐病發病狀況。本試驗共重複 6 次，其中 3 次為浸泡次氯酸鹽溶液 30 秒，另外 3 次為浸泡 60 秒。

次氯酸鹽溶液對田間自然感染果實的防治試驗

於 2022–2024 年間的 7–9 月俟紅龍果濕腐病好發季節，至紅龍果主要產區購買當天上午 6:00–8:00 左右採收之果實，包括白肉與「大紅」品種。果實來源包括位於彰化縣二林鎮的合作社 (保證責任彰化縣原斗果菜生產合作社) 收購的果實或者直接向農友 (彰化縣二林鎮、臺中市外埔區及南投縣集集鎮) 購買已裝箱的果實，於上午 11:00 前回實驗室進行處理。處理組之果實浸泡次氯酸鹽溶液 (有效氯 200 mg L^{-1}，pH 8.1–8.4) 30 秒後，放置於 24–30℃、RH > 95%、避光的環境 4–5 日後觀察濕腐病發病狀況。對照組 (未處理組) 則未浸泡次氯酸鹽溶液。每組生物重複數 9 粒以上，視當日果農採收狀況而定。

統計方法與繪圖

孢子發芽抑制率

當發芽管長度大於孢子長度1/2視為發芽。孢子發芽抑制率為 (對照組發芽率 − 處理組發芽率)/(對照組發芽率) × 100%。

菌絲生長抑制率

由於菌株 28℃下於 2 日內即長滿直徑 9 cm

之培養基，因此每皿每日生長速率計算方法為 (生長滿 1 日之菌落半徑 /1 日) × 100%。菌絲生長抑制率為 [(對照組每日生長速率 – 處理組每日生長速率)/ 對照組每日生長速率] × 100%。

罹病級數 (disease index)

分為 5 級 (0：無病徵；1：罹病面積 < 25%；2：罹病面積 > 25%，< 50%；3：罹病面積 > 50%，< 75%；4：罹病面積 > 75%，< 100%)。

發病率

計算方式為：(罹染濕腐病果實數量/全部果實數量) × 100%。防治率為：[(未處理組的發病率 – 處理組發病率)/未處理組發病率] × 100%。

統計方法

菌絲與孢子發芽抑制率方面，因為不同分離株對次氯酸鹽溶液的敏感度即具有差異，各試驗間包含分離株差異與試驗差異共 2 個變因，為瞭解次氯酸鹽的影響性，利用統計分析軟體 Microsoft Office Excel™ 2010 (Microsoft, Redmond, WA, USA) 進行雙因子變方分析 (two-way analysis of variance; two-way ANOVA)；最後以費雪最小顯著性差異法 (Fisher’s least significant difference; LSD) 測驗在 $P < 0.05$ 顯著水準下，比較處理間平均值之差異。果實罹病級數與發病率方面，係利用 JASP 0.19.3.0 版統計分析軟體 (Love *et al*. 2019) 先進行無母數單因子變異數分析之 Kruskal-Wallis 檢定，確認 $P < 0.01$ 後，再以 Dunn’s test 進行事後檢定比較處理間平均值之差異，並且以更嚴謹之 Holm 校正法取得校正後之 P 值 (P_{Holm})。按照試驗樣本的變異程度與試驗目的，檢定時採用的數值結構以及顯著水準有所差異。在人工感染防治試驗中，罹病級數的檢定方式為取每次各處理組內生物重複數之平均罹病級數為該次試驗該處理之代表數據，再以這些試驗重複之平均值進行各處理間的差異分析，以校正後的 $P_{Holm} < 0.1$ 視為具顯著差異性。在田間感染防治試驗中，分析平均罹病級數時，僅比較同一次試驗內處理組間的生物重複數據差異，$P_{Holm} < 0.05$ 作為顯著水準；發病率以各次試驗的發病率作為重複值進行統計分析。

繪圖

盒鬚圖 (box-and-whisker plot) 係以 Microsoft Office 365 中的 Excel 軟體繪製，並以 PowerPoint 協助繪製。

結果

次氯酸鹽溶液對 *Gilbertella persicaria* 菌絲與孢子抑制率

為瞭解次氯酸鹽對 *G. persicaria* 的抑制狀況，以 F218061、F218072 及 F216139 為供試菌株，測試在培養基下 (*in vitro*)，不同有效氯濃度的次氯酸鹽溶液對菌絲與孢子抑制的狀況 (表 1)。菌絲生長抑制方面，有效氯濃度 50、100 及 150 mg L^{-1} 對 *G. persicaria* 菌株的平均孢子發芽抑制率在 1.4 ± 0.9% 至 10.5 ± 7.6% 間，無顯著差異；雖然有效氯濃度 200 mg L^{-1} 的平均菌絲生長抑制率顯著高於 50 與 100 mg L^{-1} ($P < 0.05$)，然而僅 19.9 ± 7.6%，仍未超過 50%。孢子發芽抑制方面，有效氯濃度 150 mg L^{-1} 下的平均孢子發芽抑制率超過 50%，為 59.6 ± 17.3%，顯著高於 50 與 100 mg L^{-1} ($P < 0.05$)；而有效氯濃度 200 mg L^{-1} 下的平均孢子發芽抑制率為 100 ± 0.0%，顯著高於 50、100 及 150 mg L^{-1} (顯著水準為 $P < 0.05$)。

最適次氯酸鹽溶液之使用濃度與時間

為找到最適之浸泡濃度，白肉紅龍果果實於接種 *G. persicaria* 的 0.5 hpi，浸泡不同有效氯濃度之次氯酸鹽溶液 1 分鐘，觀察罹病狀況 (圖 1)。結果顯示，以有效氯 50 與 100 mg L^{-1} 處理的果實，其罹病狀況對比於對照用的水處理組與未處理組沒有顯著差異，平均罹病級數介於 1.2–2.1 間；處理以有效氯 150 與 200 mg L^{-1} 者，其平均罹病級數皆為 0.6，兩者間沒有顯著差異，而較對照組顯著降低 (顯著水準為 $P_{Holm} < 0.1$)。

為找到最適濃度與其浸泡時間，果實於接種 0.5 hpi，以有效氯濃度 100 與 200 mg L^{-1} 之次氯酸鹽溶液各自浸泡不同時間後，觀察罹病

表 1. 不同有效氯濃度次氯酸鹽溶液在培養基上對於 *Gilbertella persicaria* 的孢子發芽與菌絲生長抑制效果。

Table 1. Comparison the effect of available chlorine concentrations in hypochlorite (OCl⁻) solutions on inhibiting *Gilbertella persicaria* spore germination and hyphal growth.

Available chorine concentration (mg L^{-1})	Hyphae growth inhibition rate (%)[z]				Spore germination inhibition rate (%)[y]			
	F218061	F218072	F216139	Average	F218061	F218072	F216139	Average
50	1.3 ± 1.2	3.0 ± 1.7	-0.2 ± 1.9	1.4 ± 0.9 a	23.5 ± 3.1	18.3 ± 8.7	8.0 ± 8.0	16.6 ± 4.6 a
100	3.9 ± 0.2	8.0 ± 1.6	1.6 ± 3.5	4.5 ± 1.9 a	36.1 ± 14.0	40.7 ± 22.6	19.7 ± 19.7	32.2 ± 6.4 a
150	6.8 ± 0.7	25.0 ± 7.8	-0.4 ± 3.7	10.5 ± 7.6 ab	68.1 ± 5.1	84.3 ± 6.7	26.3 ± 21.1	59.6 ± 17.3 b
200	11.9 ± 4.6	35.1 ± 3.5	12.7 ± 3.3	19.9 ± 7.6 b	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0 c

[z] Spore germination (SG) inhibition rate was calculated as: (SG rate of treatment – SG rate of control) / (SG rate of control) × 100%. This experiment was conducted 3 times, using 500 sporangial spores of each isolate for each time. Mean ± *SE*. Different letters indicate significant differences within each group ($P < 0.05$, two-way analysis of variance (two-way ANOVA), Fisher's least significant difference (LSD) test).

[y] Hyphae growth (HG) inhibition rate was calculated as: (HG rate of treatment – HG rate of control)/HG rate of control × 100%, HG rate = (diameter of 1 d post inoculation)/1 d × 100%. This experiment was conducted 3 times, with 4 replicates of each isolate for each time. Mean ± *SE*. Different letters indicate significant differences within each group ($P < 0.05$, two-way ANOVA, LSD test).

狀況 (圖 2)。在本次試驗中，有效氯 100 mg L^{-1} 濃度下，果實浸泡 1、5 及 10 分鐘後的平均罹病級數介於 2.1–2.6，較未處理組的 3.1 與水處理的對照組 2.8 的平均罹病級數稍低，但在統計上無顯著差異；有效氯 200 mg L^{-1} 下，果實浸泡 1 分鐘的平均罹病級數降低至 1.3，顯著低於水處理與未處理對照組，而果實浸泡 5 與 10 分鐘的平均罹病級數各自微幅提升至 1.9 與 2.1，對比水處理組與未處理對照組無顯著差異。綜合前述結果，以有效氯 200 mg L^{-1} 的次氯酸鹽溶液浸泡處理 1 分鐘的果實平均罹病級數最低 (顯著水準為 $P_{\mathrm{Holm}} < 0.1$)。

為了進一步評估若將有效氯 200 mg L^{-1} 的次氯酸鹽溶液處理時間縮短在 1 分鐘內，能否有助降低罹病級數，果實於接種 0.5 hpi 各自處理有效氯 200 mg L^{-1} 次氯酸鹽溶液不同秒數後，觀察罹病狀況 (圖 3)。本試驗僅以未處理組作為對照組，平均罹病級數為 1.5，而果實浸泡 10 秒的平均罹病級數為 0.9，雖有微幅降低但與未處理組相比並無顯著性差異；果實浸泡 30 秒者平均罹病級數降至 0.4，果實浸泡 60 秒者亦降為 0.5，顯著低於對照組。結果顯示，有效氯 200 mg L^{-1} 的次氯酸鹽溶液下處理以 30 秒的果實罹病級數最低，60 秒的果實次之，但兩者結果相似，且在統計上無顯著差異 (顯著水準為 $P_{\mathrm{Holm}} < 0.1$)。

接種濕腐病後處理時間點與防治效益評估

為瞭解次氯酸防治紅龍果濕腐病的效益會不會受到感染時間長短影響，以白肉紅龍果果實供試，於接種後不同時間點處理次氯酸 200 mg L^{-1}、30–60 秒後，觀察果實罹病狀況 (圖 4)。本試驗與前面試驗結果 (圖 1、2) 不同的是，與未處理組的果實平均罹病級數 2.2 ± 0.1 相比，作為對照組之一的水處理者，於 0.5、6 及 12 hpi 之平均罹病級數亦降為 0.9 ± 0.2 到 1.4 ± 0.3 間，其中以 6 hpi 處理時罹病級數最低，雖然與未處理組在統計上沒有顯著性差異；直到 24 hpi 平均罹病級數升為 1.8 ± 0.5，與未處理無明顯差異。處理次氯酸鹽溶液方面，在 0.5、6 及 12 hpi 處理者平均罹病級數降低至 0.2 ± 0.1 到 0.5 ± 0.2 間，與未處理組相比具顯著性差異。雖然次氯酸鹽溶液處理組

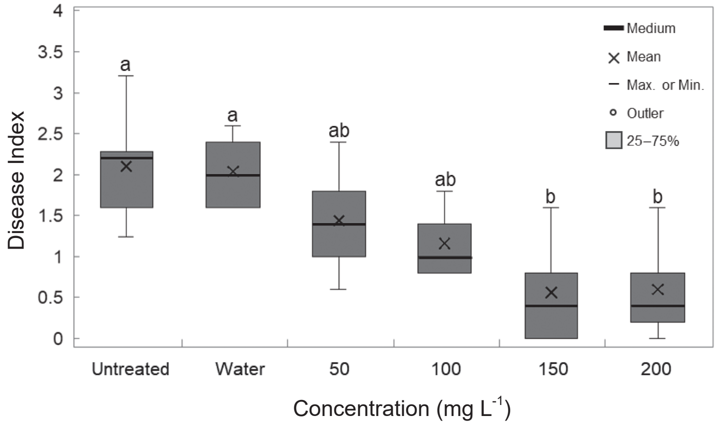

圖 1.　不同有效氯濃度的次氯酸鹽溶液對白肉種紅龍果果實濕腐病罹病級數之影響 (處理 1 分)。罹病級數 (disease index)：分為 0–4 級 (0：無病徵；1：罹病面積 < 25%；2：罹病面積 > 25–50%；3：罹病面積 > 50–75%；4：罹病面積 > 75–100%)。不同字母代表具顯著差異性 (Kruskal-Wallis 無母數檢定後，再以 Dunn's test 進行事後檢定，並以 Holm 校正法取得校正後之 $P_{Holm} < 0.1$)。

Fig. 1.　Effect of different available chlorine concentrations in hypochlorite (ClO^-) solutions on the wet rot disease index of white-flesh pitaya fruit (treated for 1 min). Disease index was rated on a 0–4 scale (0: no symptom; 1: < 25%; 2: > 25–50%; 3: > 50–75%; 4: > 75–100% diseased area). Different letters indicate significant differences among treatments (Kruskal-Wallis test followed by Dunn's test with Holm correction, $P_{Holm} < 0.1$).

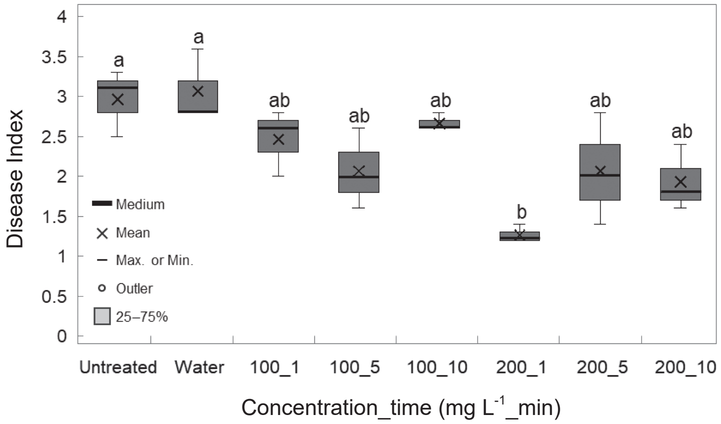

圖 2.　白肉種紅龍果以不同有效氯濃度的次氯酸鹽溶液處理不同時間 (分) 對於果實濕腐病罹病級數的影響。罹病級數 (disease index)：分為 0–4 級 (0：無病徵；1：罹病面積 < 25%；2：罹病面積 > 25–50%；3：罹病面積 > 50–75%；4：罹病面積 > 75–100%)。不同字母代表具顯著差異性 (Kruskal-Wallis 無母數檢定後，再以 Dunn's test 進行事後檢定，並以 Holm 校正法取得校正後之 $P_{Holm} < 0.1$)。

Fig. 2.　Effect of different available chlorine concentrations in hypochlorite solutions and treatment durations (min) on the wet rot disease index of white-flesh pitaya fruit. Disease index was rated on a 0–4 scale (0: no symptom; 1: < 25%; 2: > 25–50%; 3: > 50–75%; 4: > 75–100% diseased area). Different letters indicate significant differences among treatments (Kruskal-Wallis test followed by Dunn's test with Holm correction, $P_{Holm} < 0.1$).

與水處理組相比無顯著差異，但仍可看出次氯酸鹽溶液處理組罹病級數出現降低的趨勢；其中以 6 hpi 處理者最低，12 hpi 次之，0.5 hpi 再次之，然三者間在統計上無顯著差異。直到 24 hpi，次氯酸處理組、水處理組及未處理組無明顯差異。由以上結果顯示，次氯酸鹽處理的防治效益在感染 *G. persicaria* 12 小時內 (顯著水準為 $P_{Holm} < 0.1$)。

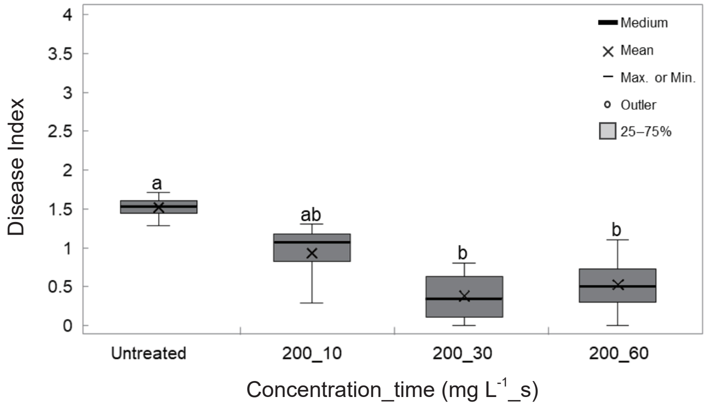

圖 3. 白肉種紅龍果以有效氯 200 mg L^{-1} 的次氯酸鹽溶液處理不同時間 (秒) 對於濕腐病罹病級數的影響。罹病級數 (disease index)：分為 0–4 級 (0：無病徵；1：罹病面積 < 25%；2：罹病面積 > 25–50%；3：罹病面積 > 50–75%；4：罹病面積 > 75–100%)。不同字母代表具顯著差異性 (Kruskal-Wallis 無母數檢定後，再以 Dunn's test 進行事後檢定，並以 Holm 校正法取得校正後之 $P_{Holm} < 0.1$)。

Fig. 3. Effect of treatment durations (s) of hypochlorite solution (available chlorine concentration of 200 mg L^{-1}) on the wet rot disease index of white-flesh pitaya fruit. Disease index was rated on a 0–4 scale (0: no symptom; 1: < 25%; 2: > 25–50%; 3: > 50–75%; 4: > 75–100% diseased area). Different letters indicate significant differences among treatments (Kruskal-Wallis test followed by Dunn's test with Holm correction, $P_{Holm} < 0.1$).

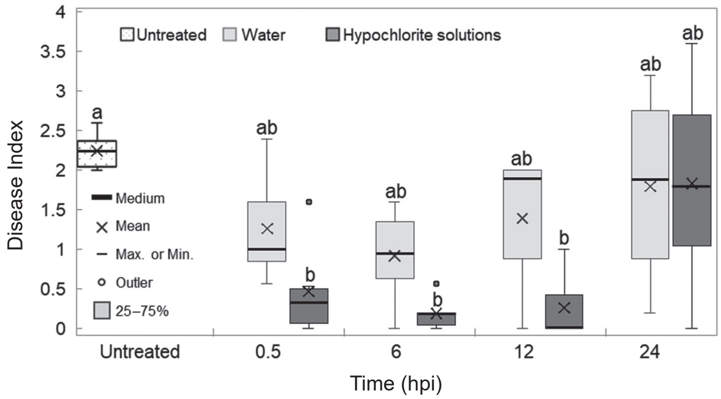

圖 4. 白肉種紅龍果在接種不同時間後，處理次氯酸鹽溶液對於果實濕腐病罹病級數的影響。罹病級數 (disease index)：分為 0–4 級 (0：無病徵；1：罹病面積 < 25%；2：罹病面積 > 25–50%；3：罹病面積 > 50–75%；4：罹病面積 > 75–100%)。不同字母代表具顯著差異性 (Kruskal-Wallis 無母數檢定後，再以 Dunn's test 進行事後檢定，並以 Holm 校正法取得校正後之 $P_{Holm} < 0.1$)。

Fig. 4. Effect of inoculation times before hypochlorite solution treatment on the wet rot disease index of white-flesh pitaya fruit. Disease index was rated on a 0–4 scale (0: no symptom; 1: < 25%; 2: > 25–50%; 3: > 50–75%; 4: > 75–100% diseased area). Different letters indicate significant differences among treatments (Kruskal-Wallis test followed by Dunn's test with Holm correction, $P_{Holm} < 0.1$).

次氯酸鹽溶液防治紅肉種果實濕腐病的效果

為瞭解前述的次氯酸施用條件是否適用在紅肉種果實上，改以紅肉「大紅」品種為供試植物，以上述最佳之次氯酸 200 mg L^{-1}、30 秒條件於 0.5–3 hpi 處理果實後觀察罹病狀況 (圖 5)。未處理組與水處理組的平均罹病級數為 1.3 ± 0.5 與 1.4 ± 0.4，次氯酸鹽溶液處理組的果實平均罹病級數微幅提升為 1.7 ± 0.2，三者

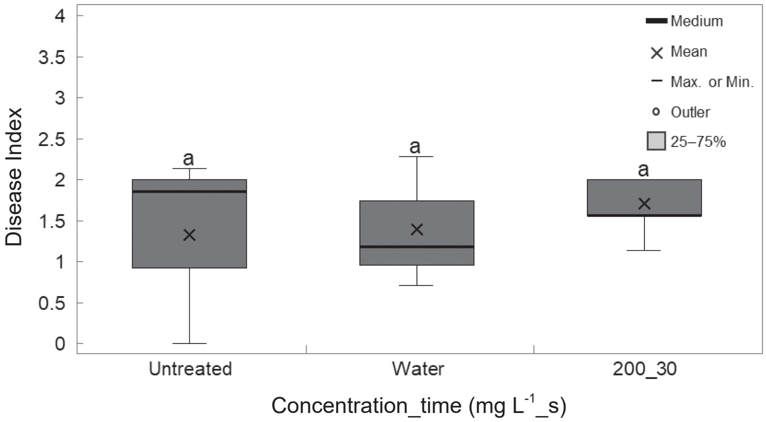

圖 5.　紅肉種紅龍果以有效氯 200 mg L^{-1} 的次氯酸鹽溶液處理後，對於果實濕腐病罹病級數的影響。罹病級數 (disease index)：分為 0–4 級 (0：無病徵；1：罹病面積 < 25%；2：罹病面積 > 25–50%；3：罹病面積 > 50–75%；4：罹病面積 > 75–100%)。不同字母代表具顯著差異性 (Kruskal-Wallis 無母數檢定後，再以 Dunn's test 進行事後檢定，並以 Holm 校正法取得校正後之 P_{Holm} < 0.1)。

Fig. 5.　Effect of treatment with a hypochlorite solution containing 200 mg L^{-1} of available chlorine on the disease index of wet rot in red-fleshed dragon fruit. Disease index was rated on a 0–4 scale (0: no symptom; 1: < 25%; 2: > 25–50%; 3: > 50–75%; 4: > 75–100% diseased area). Different letters indicate significant differences among treatments (Kruskal-Wallis test followed by Dunn's test with Holm correction, P_{Holm} < 0.1).

在統計上皆無顯著性差異 (顯著水準為 P_{Holm} < 0.1)。

從上述結果顯示，以白肉種果實為供試材料得到的次氯酸鹽處理條件對降低紅肉種「大紅」品種紅龍果感染 *G. persicaria* 的罹病級數無效，甚至會微幅增加。

次氯酸鹽溶液對田間自然感染果實的防治試驗

為瞭解實驗室所得的次氯酸鹽條件是否適用於防治田間自然感染的果實，隨機於主要產區彰化縣二林鎮取得成熟之白肉種果實進行試驗，亦取得「大紅」紅肉種果實同步測試防治效益。處理組為果實於取回當天以次氯酸 200 mg L^{-1}、30 秒處理，對照組不處理。後續果實放置在適合 *G. persicaria* 發病的環境促使濕腐病發病，直到 4–6 日後觀察罹病級數與發病率，以發病率計算防治率 (表 2)。在白肉方面試驗重複 5 次，其中有 4 次之次氯酸鹽溶液處理具防治趨勢，處理後平均罹病級數值多數顯著低於未處理組，平均發病率由 43.0 ± 6.5% 顯著降至 9.4 ± 4.5%，平均防治率 79.0 ± 11.3% (P_{Holm} < 0.05)。然而，另一次未顯示明顯防治效果，次氯酸鹽處理者與未處理者平均罹病級數相似，發病率為皆為 83.3%，防治率為 0% (顯著水準為 P_{Holm} < 0.05)。

在紅肉方面試驗重複 5 次，其中 1 次之次氯酸鹽溶液處理具防治趨勢，處理後平均罹病級數值低於未處理組的，但不顯著 (P_{Holm} > 0.05)，發病率由 83.3% 降至 58.3%，防治率 30.0%。然而，另 4 次出現對防治無效果或具負效果的趨勢，處理後平均罹病級數值皆高於未處理組的，平均發病率由 43.0 ± 20.8% 升至 69.2 ± 13.9%，惟統計上不具有顯著性差異 (顯著水準為 P_{Holm} < 0.05)。

討論

次氯酸鹽類溶液內氯存在型態受 pH 影響而動態改變，氯在 pH 2 以下以 Cl_2 形式為主；pH 2.0–7.5 間以 HOCl 形式為主，並在 pH 4.0–5.5 間達最大量；pH 7.5 以上則逐漸以 OCl^- 形式為主 (Boecker *et al*. 2023)。本研究使用的次氯酸製造機製出的溶液 pH 為 8.1–8.4 間，所得的溶液主要以次氯酸鹽 OCl^- 形式 (約 78–85%) 存在，其餘為次氯酸 HOCl (15–22%)。

表 2. 自田間或合作社隨機採集紅龍果果實施用次氯酸鹽溶液後紅龍果濕腐病的發病變化。

Table 2. Changes in disease status of pitaya wet rot after applying hypochlorite solution to fruits randomly collected from pitaya orchards or cooperative farms.

Treatment date	Source	Number of fruits (Untreated/ClO$^-$)	Average disease index[z] Untreated	Average disease index[z] ClO$^-$		Disease incidence (%)[z] Untreated	Disease incidence (%)[z] ClO$^-$	Disease incidence (%)[z] Control rate
White flesh								
With significant disease reduction								
2022/8/4	Erlin Yuandou Cooperative, farmer 1	13/13	0.6 ± 0.2 a	0.1 ± 0.1 b		46.2	7.7	83.3
2022/9/6	Erlin Yuandou Cooperative, farmer 2	22/23	0.5 ± 0.1 a	0.3 ± 0.1 a		40.9	21.7	46.9
2024/7/12	Erlin Yuandou Cooperative, farmer 3	12/12	1.1 ± 0.3 a	0.8 ± 0.1 b		58.3	8.3	85.7
2024/7/19	Erlin Yuandou Cooperative, farmer 4	15/14	0.4 ± 0.2 a	0.0 ± 0.2 b		26.7	0.0	100.0
					Average	43.0 ± 6.5 a	9.4 ± 4.5 b	79.0 ± 11.3
Without significant disease reduction								
2024/7/19	Erlin Yuandou Cooperative, farmer 5	12/12	2.0 ± 0.3 a	2.2 ± 0.3 a		83.3	83.3	0.0
Red flesh								
With significant disease reduction								
2022/9/7	Erlin farmer 1	12/12	2.3 ± 0.4 a	1.7 ± 0.4 a		83.3	58.3	30.0
Without significant disease reduction								
2022/8/4	Erlin Yuandou Cooperative, farmer 6	13/12	0.7 ± 0.3 a	1.2 ± 0.3 a		38.5	66.7	-73.3
2022/9/27	Waipu farmer	12/13	1.2 ± 0.5 a	2.1 ± 0.4 a		33.3	76.9	-130.9
2023/7/1	Erlin Yuandou Cooperative, farmer 7	11/13	2.5 ± 0.5 a	1.8 ± 0.3 a		100.0	100.0	0.0
2024/7/5	Jiji farmer	9/9	0.0 ± 0.0 a	0.7 ± 0.4 a		0.0	33.3	N/A[y]
					Average	43.0 ± 20.8 a	69.2 ± 13.9 a	-

[z] Disease index was rated on a 0–4 scale (0: no symptom; 1: < 25%; 2: > 25–50%; 3: > 50–75%; 4: > 75–100% diseased area). Disease incidence (%) = (number of infected fruits/total fruits) × 100%; Control efficacy (%) = [(incidence of control − incidence of treatment)/incidence of control] × 100%. Mean ± *SE*. Different letters indicate significant differences among treatments (Kruskal-Wallis test followed by Dunn's test with Holm correction, $P_{Holm} < 0.05$). Comparisons of average disease index were conducted only within each tests; Comparisons between white flesh and red flesh were not performed.

[y] Not applicable. This data was not included in the average of control rate.

製造機使用的原料為食鹽 NaCl，因此溶液內含有 Na^+，可能與 OCl^- 結合成 NaOCl (次氯酸鈉)。目前關於『次氯酸水或次氯酸鹽殺菌劑』名稱在國內仍未有共識 (Wang 2020)，本研究以『次氯酸鹽溶液』作為稱呼，並以測定之有效氯濃度來表示次氯酸鹽 OCl^- 濃度高低。

有效氯濃度在 200 mg L^{-1} 以內對於 *G. persicaria* 菌絲的生長抑制效果低，但濃度在 150 mg L^{-1} 以上的次氯酸鹽溶液處理對 *G. persicaria* 孢子發芽抑制約略高於 50%，而 200 mg L^{-1} 以上即可達 100% 抑制效果 (表 1)。抑制孢子發芽為防治紅龍果濕腐病的關鍵之一 (Lin *et al*. 2020)，因此次氯酸鹽溶液具有防治紅龍果濕腐病的潛力，惟因為對菌絲抑制效果不明顯，因此需要在感染前期使用。

本研究中，白肉種果實各別浸泡在 100 與 200 mg L^{-1} 以上的次氯酸鹽溶液時間越久，例如各別超過 10 與 5 分鐘以上，罹病級數反而提高 (圖 2)；本實驗室在其他試驗中的觀察也發現，果實浸泡在次氯酸鹽溶液的時間越久，冷藏 4 週後越容易出現表皮褐化斑點等類似傷害現象 (未顯示的資料)。另外，在 Lin *et al*. (2014) 研究顯示，傷口有助於 *G. persicaria* 感染紅龍果果實。推測果實在接觸到不適當的次氯酸鹽條件時 (如濃度或處理時間不適當)，可能在果實表面產生更多的傷害；例如造成微型的傷口，讓未被次氯酸鹽除盡的殘留 *G. persicaria* 病原菌更易入侵。雖然高濃度的次氯酸鹽溶液可抑制 *G. persicaria* 菌絲生長與孢子發芽 (表 1)，但也需考慮對果實的影響性，浸泡高濃度與長時間的次氯酸鹽溶液可能對防治紅龍果濕腐病反而適得其反。

本研究顯示，在 12 hpi 內的施用次氯酸鹽溶液有較佳的防治效果 (圖 4)，此可能跟病原菌的發展進程有關。真菌類病原菌以孢子狀態成功於植物體內建立感染的階段大致可分為『附著 (attachment of pathogen to host)』、『孢子發芽 (spore germination)』、『入侵 (penetration)』、『菌絲生長 (growth)』及『建立族群 (colonization)』(Agrios 2005)，時間長短視病原菌特性而異。雖然目前 *G. persicaria* 侵染植物體的顯微病程發展尚未清楚，但紅龍果濕腐病從果實接種到發病僅需 3–4 日 (Lin *et al*. 2014)，屬發病較快的病害種類。已知次氯酸鹽僅對 *G. persicaria* 孢子具較高的抑制效果 (表 1)，而在 12 hpi 前施用次氯酸鹽才具備良好防治效果 (圖 4)，由此推測 12 hpi 以前是孢子發芽侵染果實的關鍵時刻；次氯酸鹽對菌絲抑制效果不佳，而在 24 hpi 施用次氯酸鹽的防治效果不佳，推測 24 hpi 病原菌可能已侵入紅龍果果實內，甚至並開始出現菌絲生長，此時次氯酸鹽除了無法抑制菌絲生長外，可能也無法深入果皮內殺菌。

從人工接種試驗可發現 (圖 1、2)，0.5 hpi 後施用水仍與未處理 (對照組) 的罹病級數相似。顯示即便水沖刷，後續病勢發展亦不受明顯干擾，*G. persicaria* 的感染在 0.5 hpi 即建立。然而，不同的是，在探討感染時間長短與次氯酸鹽溶液的防治效果差異中 (圖 4)，與未處理相比，水處理之罹病級數亦出現降低的趨勢。推測原因之一或許是菌株在長期培養基條件下繼代培養發生異常，如造成附著果實表面的能力衰退，以致水沖刷即脫落果表；但本試驗中未處理組的罹病級數及發病時間與先前試驗的結果相似，顯示後續的入侵以及建立族群的能力不受影響。另外，在本試驗中 (圖 4)，雖然『在 12 hpi 前處理次氯酸鹽溶液者，罹病級數較水處理組降低』的趨勢存在，然而次氯酸鹽處理組與水處理組間在統計上不若先前的試驗有顯著差異性 (圖 1、2)。其原因除了上述討論的菌株狀況外，亦可能為每批次的接種條件微幅差異，如接種室溫溼度或果實成熟度等，造成各次罹病狀況差異幅度大，導致數據不顯著。

在 6 hpi 處理次氯酸鹽溶液者罹病級數最低，雖然與 0.5 與 12 hpi 的罹病級數在統計上沒有顯著性差異；根據未顯示的資料，*G. persicaria* 的孢子在 24–28℃常溫 PDA 培養基下於 7 時以內孢子發芽率即達 50–90%。推測 6 hpi 前後是孢子發芽並轉換成入侵的重要階段，次氯酸除了直接抑制孢子發芽外，或許在抑制入侵階段扮演關鍵角色。關於 *G. persicaria* 的發病過程若能有更多的瞭解，應越有助於發展合適的防治策略，未來值得進一步探討。

在人工感染防治試驗中，雖然每批次間的果實與感染條件有些微差異，但較田間自然感染者條件相對一致，試驗變因僅為處理與否等單一因子。因此，取各處理組內生物重複數之平均罹病級數為單次試驗代表數值，以降低個體差異，並且重複 3 次試驗以上取得重複數值進行統計檢定。在試驗中，人工接種的病原菌量高，且自接種開始即給予合適的發病環境，對照組與處理組的發病率多數可達近 100%。在此人為的極端高發病壓力下，為最大篩選出具潛在防治效果之處理組，僅採罹病級數探討各處理對於發病嚴重的影響性，且以 $P_{Holm} < 0.1$ 視為具顯著差異性。在田間自然感染防治試驗中，除試驗變因可控外，其他各批次的果實樣本其栽培環境與季節，尤其田間感染狀況差異程度大，如感染時間長短不同、病原接種量高低不均一、田間不同病原菌之病原性強弱不同及感染時環境條件各異等，各批次果實樣本整體變異度大。因此在分析平均罹病級數時，則改以增加生物重複數至 9–23 以降低個體差異的影響，且僅比較同一批次處理組間的差異，未以各批次間的平均值差異進行統計檢定，以排除果實樣本差異的干擾，並以 $P_{Holm} < 0.05$ 作為顯著水準，以符合一般學術慣例。至於發病率的統計方式則受限於每次試驗僅可得單一代表值，因此仍以各次試驗的發病率作為重複值，取得各次發病率後比較各組平均發病率的差異。紅龍果濕腐病可造成果實腐爛且發病快速，果實一旦發病幾乎失去商品價值。因此，評估防治效果的高低時，相較於罹病級數應以發病率高低更符合實際需求。觀察田間自然感染防治試驗的發病率，可知各地的發病率不若人工感染防治試驗中的高，顯示本研究中的人工接種確實為極端的狀況；另外，白肉果實經過次氯酸鹽溶液處理後，不僅『罹病級數』降低，更重要的防治指標『發病率』亦出現降低情形，確認透過本研究篩選方式所得的防治條件適用於田間自染感染者，可達到防治效果。

田間自然感染防治研究中的果實，是隨機取自當天早上送往合作社的果實，或是取自特定農民於個人集貨場裝箱之果實，隨後帶回實驗室進行次氯酸鹽溶液處理，經過一段時間後觀察罹染濕腐病的狀況，其他病害發病狀況暫忽略不計。白肉種採後處理的 5 次試驗中，僅出現 1 次無防治效果 (表 2)。該次係蒐集自 2024 年 7 月 19 日於二林原斗合作社農友，對照組的罹病級數與發病率為 2 ± 0.3 與 83.3%，在 5 組的試驗中屬罹病狀況較嚴重的一次。關於罹病狀況嚴重是否會降低次氯酸鹽類的防治效果，根據本研究的人工接種試驗，未處理的對照組罹病級數自 1.5 ± 0.1 至 3.0 ± 0.2 時 (圖 1–4)，發病率近 100% (資料未顯示)，施用合適條件的次氯酸鹽溶液的處理組仍具良好降低罹病級數之效果。因此在田間高罹病級數與高發病率之狀況下，次氯酸鹽溶液防治效果是否會失效仍有待確認。另外，在接種時間長短與防治效果的試驗中可知，在 12 hpi 內處理次氯酸則防治效果佳；若 24 小時後才處理次氯酸則防治效果差 (圖 4)。病原菌 *G. persicaria* 在田間的主要感染時期為果實成熟後期，特別是採收時，果實容易因為人為採收作業出現傷口，而促進 *G. persicaria* 感染 (Lin *et al.* 2025)。按照農民習慣，送合作社運銷的果實常係當天或前一日採收的果實，此時的果實距感染可能已超過 12 小時。因此，該次不良的防治效果原因亦不排除可能跟該果園的果實罹病時間較久有關。

從白肉種果實得到的最適次氯酸鹽處理條件，於「大紅」紅肉種果實上測試防治的效果試驗時。得知在人工接種 *G. persicaria* 的測試中，與對照組 (包括未處理組與水處理組) 相比，次氯酸鹽處理過的果實罹病級數並未出現降低趨勢或顯著差異 (圖 5)。田間採後處理結果也顯示此條件對紅肉種效果不佳，試驗重複 5 次，僅 1 次具防治效果，其他 4 次甚至出現罹病狀況反而更嚴重的趨勢。推測病害變嚴重的原因，可能跟不適當的次氯酸鹽濃度與時間傷害紅肉種果實表面有關。當果實接觸到氯系藥劑，體內呼吸及其他生理代謝亦會改變 (Guo *et al.* 2013)。比較紅肉種與白肉種果皮的性質，紅肉種果皮的酚類 (phenolics)、黃酮類化合物 (flavonoid) 含量以及整體的抗氧化能力較白肉種的高。然而，特別的是，白肉種果實

表皮卻有較高的維他命 C 含量 (Senadheera & Abeysinghe 2015)。其中，維他命 C 除可保護細胞抵禦因次氯酸引起的傷害，還能調節次氯酸在細胞內的作用，從而影響生理與病理上的發展 (Carr *et al*. 2000)。紅肉種與白肉種的表皮成分差異大，紅肉種較白肉種不耐次氯酸鹽處理的原因仍有待確認。綜合以上結果，以白肉種測得之次氯酸鹽條件不適用於紅肉種。紅肉種紅龍果是否適用次氯酸達到防治或延長櫥架衰退期的時間，仍需另外評估。

本研究發現白肉紅龍果適用的次氯酸鹽溶液其有效氯濃度高達 200 mg L^{-1} (pH 8.1–8.4 下)，最適合處理時間在 30–60 秒內，較其他研究試驗屬高濃度與短時間。例如，Tang (2022) 在降低金柑腐損率的試驗中，係處理以有效氯濃度較低的 40 mg L^{-1} 次氯酸水溶液，且浸泡時間為 5 分鐘。本研究中有效氯 50 mg L^{-1} 的次氯酸鹽溶液處理 1 分鐘下，對紅龍果濕腐病防治效果有限 (圖 1)，而在 100 與 200 mg L^{-1} 下延長處理時間可能出現反效果 (圖 2)；然而，低濃度 (如 50 mg L^{-1} 以下) 之次氯酸鹽溶液延長處理時間是否能達到抑制紅龍果濕腐病的效果尚未經測試，是未來可嘗試的方向。另外，本研究使用的次氯酸鹽溶液 pH 為 8.1–8.4 間，溶液內主要的有效氯係殺菌能力較次等的 OCl^- 形式，而更強的殺菌物質 HOCl 形式係在 pH 4–5.5 間達最高濃度 (Boecker *et al*. 2023)。未來可朝調整降低 pH 值以增加溶液的 HOCl 形式，進而增加潛在殺菌能力，期藉此降低使用之次氯酸鹽溶液濃度。

本研究為首次探討次氯酸鹽溶液用於防治 *G. persicaria* 引起的紅龍果採後濕腐病的報告，確定針對白肉種紅龍果的處理條件與限制。研究顯示，若白肉種果實在接種 *G. persicaria* 的 12 小時內及時處理有效氯 200 mg L^{-1} 的次氯酸鹽溶液 (pH 8.1–8.4) 30–60 秒，能顯著降低罹病級數。進一步自集貨場蒐集果實的採後處理結果，更顯示此次氯酸鹽條件具穩定的防治效果；即便錯過最適時間導致防治效果不佳，亦不會對果實造成負面影響。建議在病原菌可能附著果實後，儘速於 12 小時內施用次氯酸鹽溶液，以達更好的防治效果。

誌謝

本研究承蒙農業部科技計畫 114 農科 -15.1.1-農-02 計畫經費支持；感謝林依佳與詹月梅擔任研究助理協助完成、農業試驗所作物組楊滿霞博士提供生物統計諮詢、保證責任彰化縣原斗果菜生產合作社許惠萍主席協助蒐集果實，謹此致謝。

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Evaluation of the Effectiveness of Hypochlorite Solution in Controlling Postharvest Wet Rot of Pitaya Fruits

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Abstract

Lin, C. P., C. W. Huang, J. H. Huang, J. N. Tsai, and M. C. Hsu. 2026. Evaluation of the effectiveness of hypochlorite solution in controlling postharvest wet rot of pitaya fruits. J. Taiwan Agric. Res. 75(1):103–116.

Pitaya (*Hylocereus* spp.) is an economically important fruit crop, and wet rot disease caused by *Gilbertella persicaria* has become a major concern to the industry. This study aimed to evaluate the efficacy of hypochlorite solution (pH 8.1–8.4) in controlling postharvest wet rot disease of pitaya fruit. In an *in vitro* assay, hypochlorite solution with available chlorine concentrations up to 200 mg L^{-1} didn't significantly inhibit mycelial growth of *G. persicaria*. In contrast, spore germination was effectively suppressed at concentrations above 150 mg L^{-1}. Further artificial inoculation tests using fruits of white-fleshed pitaya cultivar revealed that postharvest treatment with 200 mg L^{-1} hypochlorite solution for 30–60 s significantly reduced the disease index (rated on a 0–4 scale based on the diseased area) of wet rot disease. Evaluations of treatment timing indicated that application within 12 h after inoculation significantly reduced the disease index, whereas treatments applied after 24 h showed no significant difference compared with the untreated control. Notably, with the same treatments, no protective effect was observed on red-flesh pitaya cultivar artificially inoculated with the pathogen. Postharvest treatments of naturally infected fruits under field conditions revealed that application of hypochlorite solution containing 200 mg L^{-1} chlorine reduced the average disease incidence of wet rot in white-flesh pitaya from 43.0 ± 6.5% in untreated fruits to 9.4 ± 4.5%, achieving a control efficacy of 79.0 ± 11.3%. In contrast, the same treatment unexpectedly increased disease incidence in red-flesh pitaya, with the average incidence rising from 43.0 ± 20.8% in untreated fruits to 69.2 ± 13.9%. Overall, fruit postharvest treatment with hypochlorite solution effectively reduces wet rot disease index in white-fleshed cultivar, however, alternative management strategies are required for red-fleshed cultivars.

Key words: *Gilbertella persicaria*, Postharvest treatment, Optimal concentration and duration, Timing of treatment.

Received: September 5, 2025; Accepted: October 30, 2025.

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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):117–134 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0007

Research Article

Challenges for Sustainable Pest Management Utilizing Indigenous Natural Enemies in Chrysanthemum Fields in Okinawa

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Abstract

Ganaha-Kikumura, T. and A. Honma. 2026. Challenges for sustainable pest management utilizing indigenous natural enemies in chrysanthemum fields in Okinawa. J. Taiwan Agric. Res. 75(1):117–134.

Thrips nigropilosus is the most economically important pest of chrysanthemum. To manage this species, there is a need for integrated pest management (IPM), including the use of natural enemies on chrysanthemums, as an environmentally friendly and sustainable method of pest control. We conducted the following investigations to develop a technique for utilizing indigenous natural enemies of this pest in chrysanthemum: (1) exploration of natural enemies of *T. nigropilosus* in chrysanthemum fields; (2) evaluation of the predation ability of a potential natural enemy, *Campylomma livida*, against *T. nigropilosus*; and (3) field trials to assess the effectiveness of augmentative release methods for *C. livida*. In terms of the identified natural enemies, *Campylomma* spp., *Geocoris ochropterus*, *Orius* spp., *Haplothrips* spp., and *Ceranisus* sp. were found in chrysanthemum fields, with *C. livida* being the most abundant. In the laboratory, we observed that an adult *C. livida* could feed on at least 9.0 adults and 9.9 larvae of *T. nigropilosus* in 24 h. Therefore, a field trial was conducted using *C. livida* to examine its effects on lowering the density of *T. nigropilosus* and reducing the damage it causes. Consequently, the number of plants damaged by thrips decreased to one-fifth in the biological control plot (*C. livida* released), compared with the findings in the conventional plot where pesticides were sprayed regularly or to the control plot where no pesticides were sprayed. Damage to chrysanthemum was also reduced to less than one-third in the biological control plot, providing evidence for *C. livida*'s effectiveness for managing thrips in chrysanthemum fields.

Key words: *Thrips nigropilosus*, Ryukyu, Biological control, *Campylomma livida*.

INTRODUCTION

The regular application of synthetic pesticides has significant impacts on ecosystems (Goulson 2013; Chagnon *et al*. 2015) and facilitates the development of pesticide resistance in pest populations. For example, *Frankliniella occidentalis* and *Tetranychus urticae*, which are important pests of chrysanthemum (de Jager *et al*. 1995; Ganaha-Kikumura *et al*. 2012; OPPPC 2018), have developed a high degree of resistance to various pesticides (Osakabe *et al*. 2009; Ohno *et al*. 2010), resulting in control measures against these pests becoming inadequate. The extensive use of ineffective pesticides is also laborious and expensive for growers and is particularly

Received: May 7, 2025; Accepted: November 19, 2025.
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undesirable in Japan, where the population engaged in agriculture is aging.

In Japan, 59 billion yen worth of *Chrysanthemum morifolium* Ramat (Asteraceae) was produced in 2023, with Okinawa accounting for 10% of that (MAFF 2024). Chrysanthemum cultivation in Okinawa Prefecture is conducted almost yearly and December and March are the main shipping periods to meet high demand, as chrysanthemum can be produced in Okinawa at a lower cost than in other Japanese regions by taking advantage of the mild winter climate and growing in open fields. The most important pests of chrysanthemum in Okinawa are thrips, spider mites, aphids, bugs, and lepidopteran larvae, with *Thrips nigropilosus* (Thysanoptera: Thripidae) being the most important (Ganaha-Kikumura *et al*. 2012). *T. nigropilosus* is widely distributed all over the world (Mound 2010) and is known to attack a variety of crops (Sakimura 1939; Bullock 1965; Stannard 1968; Mound *et al*. 1976; Umeya & Okada 2003).

However, *T. nigropilosus* has been regarded as a minor pest since the widespread use of pesticides reduced its pest status (Walker & Michaux 1989), and most reports on this species as a pest were published more than 40 years ago. Moreover, Ganaha-Kikumura *et al*. (2014) demonstrated that organophosphates, carbamate, synthetic pyrethroids, neonicotinoids, and other pesticides were highly effective against Okinawan populations of *T. nigropilosus*. However, in farmers' fields, the control of thrips has not been as effective as expected based on pesticide efficacy. Kijima *et al*. (2014) suggested that the cultivation of chrysanthemums in densely planted arrangements in Okinawa, where 18,000–22,000 plants are planted per 1,000 m^2, prevents the sprayed chemical solution from reaching the microhabitat of the pest, such as the underside of leaves of plants grown inside the ridges. There is thus a need to establish an integrated pest management (IPM) technique, regardless of pesticide resistance.

Various control methods that can be incorporated into IPM approaches against thrips have been evaluated for their effectiveness. Recently, it was reported that red light irradiation of plants changes the behavior of *Thrips palmi*, with fewer individuals being attracted to plants irradiated with red light than to plants subjected to normal light illumination (Murata *et al*. 2018). In addition, Ganaha-Kikumura & Zakimi (2018) reported that irradiation with red LED lights was significantly effective at lowering the density of *T. nigropilosus*, but was insufficient for the reduction of damage to chrysanthemum leaves. Because *T. nigropilosus* has been documented to attack a variety of plants, the population may be suppressed by controlling weeds around chrysanthemum fields that act as hosts of it. However, a survey of various weed species in areas in Okinawa where horticultural crops are cultivated showed that *T. palmi* was the dominant species, while *T. nigropilosus* was only found on a few Asteraceous weeds in chrysanthemum fields (Ganaha-Kikumura *et al*. 2022). In addition, netting houses, which have been primarily used for growing chrysanthemum to mitigate typhoon damage since 2000, have been shown not a function to reduce the number of thrips intruding into them (Ganaha-Kikumura *et al*. 2012). It thus seems to be more important to control the population density of *T. nigropilosus* in chrysanthemum fields, rather than that of individuals invading such netting houses.

In this study, we aimed to develop a technique for utilizing indigenous natural enemies of the thrips, which has rarely been attempted, for the establishment of an effective IPM system in chrysanthemum production. We conducted the following investigations: (1) exploration of potential natural enemies in open fields of chrysanthemum; (2) evaluation of the predation ability of one of the promising natural enemies, *Campylomma livida* (Hemiptera: Miridae), against *T. nigropilosus*; and (3) field trials to assess the effectiveness of augmentative release methods for *C. livida*.

MATERIALS AND METHODS

Search for indigenous natural enemies in chrysanthemum

We set the survey period in chrysanthemum fields in Okinawa Island as May–June in 2023 and 2024 for two reasons: (1) In Okinawa, blooming chrysanthemum flowers are present in the fields only during April and June since flowering is completely controlled by using electric night lights. (2) The fields that have not been sprayed with pesticides for a period of time are limited in those seasons. In total, we investigated 27 fields in the northern, central, and southern parts of Okinawa Island.

To search for natural enemies of chrysanthemum pests, 10 fully opened chrysanthemum flowers per field were collected in 70% ethanol. In some fields (n = 15) where the chrysanthemum leaves were vibrant, we collected phytophagous thrips and potential candidates of natural enemies against the thrips by beating the plants by hand ten times at each of the three locations in the field. The insects that fell onto a tray (20 cm × 60 cm, covered with Tyvek® 700AG sheet; DuPont™, Wilmington, DE, USA) were immediately stored in 70% ethanol. Potential candidates, such as *Campylomma* spp., Geocorid species (Hemiptera: Geocoridae), and *Orius* spp. (Hemiptera: Anthocoridae), were identified based on Rural Culture Association Japan (2016), as well as the reports of Yasunaga *et al*. (2001, 2015), Oida (2009), and Ishikawa *et al*. (2012). For phlaeothripid thrips, specimens were macerated with 10% KOH and mounted in Hoyer's medium or Canada balsam after dehydration in ethanol and clearing in clove oil. Species identification was performed based on the report of Okajima (2006). If a female of either of the two morphologically closely related species, *Haplothrips brevitubus* and *Haplothrips chinensis*, was found, we treated it as *H. chinensis* because *H. brevitubus* has never been reported in Okinawa (Okajima 2006; Ganaha-Kikumura *et al*. 2022). For thripid thrips, the specimens were prepared in the same way as for the phlaeothripid ones. Species identification was based on the reports published by Wilson (1975), Palmer (1992), Mound & Kibby (1998), and Masumoto & Okajima (2004). Some specimens were identified based on the work of Chiwaki *et al*. (1994) and Ganaha-Kikumura & Kawamura (2020) without specimen preparation because they had characteristic morphologies, such as for *Frankliniella cephalica*, *Microcephalothrips abdominalis*, and *T. nigropilosus*.

Evaluation of the predatory ability of the indigenous natural enemy, *C. livida*, against the chrysanthemum thrips

In accordance with the procedure described by Nakao (1993), a disk of chrysanthemum leaves (20 mm in diameter) was placed on the bottom of a cylindrical plastic container (inner diameter 18 mm, height 49 mm) and fixed with Parafilm®. Ten adults or second-instar larvae of *T. nigropilosus* were placed in the container, and the top of the container was sealed with Parafilm® soon after the release of a single adult *C. livida* that had been deprived of food for 24 h. The containers were placed in a 14L : 10D thermostatic chamber at 25℃, and the number of dead thrips was counted 24 h after releasing the predacious bug. As a control, containers without the bug were also established. In the experiments on adults, 5 replicates and 3 control replicates were performed. Meanwhile, in the experiments on larvae, 16 replicates and 3 control replicates were performed.

Trial of *C. livida* release in chrysanthemum fields

In September 2023, chrysanthemums (variety: "Kinshu") were planted in nine netting houses (2.5 m × 6 m) at Okinawa Agricultural Research Center, Itoman City. These nine houses were divided into three treatment plots as follows: (1) a predatory bug, *C. livida*, was introduced to

manage pests (hereafter, biological control plot); (2) chemical pesticides were applied regularly (hereafter, conventional plot); and (3) neither chemical pesticides nor natural enemies were applied (hereafter, control plot). In the biological control plot, one adult of *C. livida* per m^2 was released twice at a 2-wk interval. The release density was based on that of *Orius strigicollis*, a species that is commercially available in Japan as a biological control agent against thrips and that shares several biological characteristics with *C. livida*. Two holy basil plants were planted per netting house as insectary plants for the bugs. Each house was randomly assigned within an 800 m^2 field, ensuring a minimum distance of 3.0 m between them. To prevent indigenous natural enemies from intruding into the houses, broad-spectrum pesticides were sprayed around the houses at least once a week. In addition, to minimize potential spatial bias, the three treatments were systematically allocated across the houses. The insect pests and their natural enemies were surveyed at 5–12 d intervals, each covering 30 plants (90 leaves) per house. Chrysanthemums were harvested on December 14. The cut flowers were trimmed to a length of 70 cm and the leaves on the lower 20 cm of the plant were removed to meet standards for shipping. Among the remaining leaves, the number of damaged leaves was counted. For the plants that exhibited core stoppage symptoms, as caused by *Taylorilygus apicalis* (Hemiptera: Miridae), the number of sites of damage was also counted.

Statistical analysis

To evaluate the difference in the abundance of thrips among the three treatments (biological control plot, conventional plot, and control plot), we conducted statistical analysis using two hurdle models since the dataset comprised a substantial number of zeros. For both models, a binomial distribution with a logit link was used to model the absence or presence of thrips, after which Poisson and negative binomial distributions with a log link function were used for the count for each model. As a result of comparing the fitting of the two hurdle models by performing a likelihood ratio test, we chose the model with a negative binomial distribution. The dispersion parameter [Log(θ)] was also significant (estimate = -1.19, P = 0.00020), confirming the appropriateness of the model. A Kruskal-Wallis test was conducted to examine the significance of differences in spider mite and scale insect occurrences across the three treatments. Because almost all of the data were 0 in some treatments, we assessed normality using the Shapiro-Wilk test. As the data deviated from normality, we used the non-parametric Kruskal-Wallis test, followed by pairwise Wilcoxon rank-sum test with Bonferroni correction applied to adjust for multiple comparisons.

The effect of treatment on the number of damaged leaves caused by thrips per plant was analyzed using a generalized linear mixed model (GLMM). This model included treatment as a fixed effect and the netting house as a random effect. The total number of leaves per plant was included as an offset to standardize the response variable to a per-plant scale. The analysis was performed using the "glmmTMB" package in R, assuming a Poisson distribution with a log link function.

The effect of treatment on the number of sites damaged by *T. apicalis* per plant was analyzed using GLMM assuming a negative binomial distribution with a logit link function.

To test the overall significance of the treatment effect, a type II Wald chi-square test was conducted using the "ANOVA" function in the "car" package. Pairwise comparisons between treatments were performed using Tukey's method for multiple comparisons with the "emmeans" package.

Comparison of net production value

To examine the management implications of reducing the number of pesticide applications through the release of *C. livida*, the net production value was calculated by subtracting the cost of materials and labor for chemical application from the production value. The unit price of the pesticides was calculated from the retail price as of December 2024, and the time required for

pesticide/natural enemy application based on the amount of pesticide, the number of units shipped per unit area, and the unit sales price were calculated based on the regional crop-specific management indicators (OPG 2018). Since *C. livida* is not available on the market in Japan, the unit sales price of *O. strigicollis*, which is already commercially available as a natural enemy formulation, was used as a reference to calculate the unit sales price. The time required to apply *C. livida* was set to 15 min 1,000 m^{-2}; this setting was based on the standard release density of *O. strigicolli* in Japan, with which the release density of *C. livida* was aligned in the present study. The presence or absence and extent of thrips damage have a major impact on the unit price of chrysanthemums. However, there are no fixed standards for the difference in price due to insect damage because the unit price is greatly influenced by market trends and the balance of supply and demand at any given time. Therefore, in this study, the unit prices of high- and medium-grade products were calculated as 25 yen and 12 yen, respectively, by considering the plants with 0–19% and ≥ 20% damaged leaves as those of high and medium grade, respectively.

RESULTS

Search for indigenous natural enemies in chrysanthemum

Table 1 shows the results of our survey of chrysanthemum flowers. The candidate natural enemies found were *Campylomma* spp., *Orius* spp., *Haplothrips* spp., and parasitoid wasps including the genus *Ceranisus* sp. (Hymenoptera: Eulophidae) with herbivorous thrips. Except parasitoids, *Campylomma* spp. were the most abundant in terms of both the number of locations and the number of individuals found and they included two species, *C. livida* and *C. lividicornis*. We could not identify *Orius* spp. at the species level due to a lack of adult specimens. We collected three individuals of phlaeothripid thrips, two *Haplothrips chinensis*, and one *Haplothrips gowdeyi*. We collected 897 thripid thrips in the 27 fields. A total of 347 individuals were identified as *F. cephalica*, *Frankliniella intonsa*, *M. abdominalis*, *Thrips hawaiiensis*, *T. nigropilosus*, *T. palmi*, and *T. tabaci*. Among them, *F. intonsa* and *M. abdominalis* were the most abundant species, while *T. nigropilosus* was relatively uncommon (7.5% of the total). The remaining 549 individuals were not identified due to their immaturity, while one specimen was in a poor condition.

Table 2 shows the results of the survey based on beating the plants in the chrysanthemum fields 10 times. We performed investigations in 15 fields in various localities on Okinawa Island and identified two candidate natural enemies: *Campylomma* spp. and *G. ochropterus*. *Campylomma* spp. were the more common of these and were found in all surveyed areas. Twelve individuals were identified as *C. livida*, whereas *C. lividicornis* was not found in this survey. Larvae of this genus were often collected sympatrically with its adults. We collected 337 thripid thrips from the 15 fields. A total of 89 individuals were identified as *F. cephalica*, *F. intonsa*, *M. abdominalis*, *T. hawaiiensis*, *T. nigropilosus*, and *T. palmi*. Compared with the flower survey results, the proportion of *T. nigropilosus* was higher, accounting for 34.7% of the total.

Evaluation of the predatory ability of the indigenous natural enemy, *C. livida*, against chrysanthemum thrips

Despite the limited sample size, the outcome was unambiguous- both adult and larval thrips offered were almost entirely preyed upon. The adults of *C. livida* preyed on an average of 9.0 adult thrips (range 7–10 individuals) and an average of 9.9 larval thrips (range 9–10) per 24 h. No thrips died in either the adult or the larval controls.

Trial of *C. livida* release in the field

Figure 1 shows the numbers of *T. nigropilosus* and *C. livida* per shoot. *C. livida* was established on all three biological control plots throughout

Table 1. Species composition of thripid thrips and candidate natural enemies against thrips collected from chrysanthemum flowers on Okinawa Island in 2023–2024.

Area	NS[z]	Number of thripid thrips							Number of fields where natural enemies were found (number of individuals)											
									Campylomma spp.[x]						*Haplothrips* spp.				
		Total	*Frankliniella intonsa*	*Microcephalothrips abdominalis*	*Thrips nigropilosus*	*Thrips palmi*	Others[y]	Immature stages	Total	*C. livida*	*C. lividicornis*	Immature stages	Geocorid species	*Orius* spp.	Total	*H. chinensis*[w]	*H. gowdeyi*	Immature stages	Parasitoid wasps[v]
Okinawa Island																			
Northern region	8	206	46	15	10	2	5	128	1 (1)	0	0	1 (1)	0	0	1 (1)	1 (1)	0	0	1 (1)
Central region	11	417	73	98	13	3	11	219	0	0	0	0	0	0	1 (1)	0	1 (1)	0	4 (5)
Southern region	8	274	35	7	3	5	22	202	3 (4)	1 (2)	1 (1)	0	0	2 (5)	0	0	0	0	1 (1)
Total	27	897	154	120	26	10	38	549	4 (5)	1 (2)	1 (1)	1 (1)	0	2 (5)	2 (2)	1 (1)	1 (1)	0	6 (7)

[z] NS: Number of fields surveyed. All fields surveyed in the same year were at least 200 m apart.
[y] The category "Others" includes the following species: *Frankliniella cephalica, Thrips hawaiiensis*, *Thrips tabaci*, and an unidentified individual.
[x] Species identification was performed using only adult males.
[w] Data include male specimens only.
[v] Potential thrips-parasitizing wasps including the genus *Ceranisus*.

Table 2. Species composition of candidate natural enemies against thrips collected by beating chrysanthemum plants on Okinawa Island during 2023–2024.

		Number of thripid thrips							Number of fields where natural enemies were found (number of individuals)										
									Campylomma spp.[x]						*Haplothrips* spp.				
Area	NS[z]	Total	*Frankliniella intonsa*	*Microcephalothrips abdominalis*	*Thrips nigropilosus*	*Thrips palmi*	Others[y]	Immature stages	Total	*C. livida*	*C. lividicornis*	Immature stages	*Geocoris ochropterus*[w]	*Orius* spp.	Total	*H. chinensis*	*H. gowdeyi*	Immature stages	Parasitoid wasps[v]
Okinawa Island																			
Northern region	2	3	0	0	3	0	0	0	2 (4)	0	0	1 (2)	0	0	0	0	0	0	0
Central region	8	154	17	35	9	0	5	88	5 (17)	5 (6)	0	1 (2)	1 (2)	0	0	0	0	0	0
Southern region	5	180	4	0	14	1	1	160	4 (12)	4 (6)	0	2 (3)	1 (1)	0	0	0	0	0	0
Total	15	337	21	35	26	1	6	248	11 (33)	9 (12)	0	4 (7)	2 (3)	0	0	0	0	0	0

[z] NS: Number of fields surveyed. Almost all fields surveyed were included among the fields in Table 1.
[y] The category “Others” includes *Frankliniella cephalica* and *Thrips hawaiiensis*.
[x] Species identification was performed using only adult males.
[w] The central region contained a larval stage.
[v] Potential thrips-parasitizing wasps.

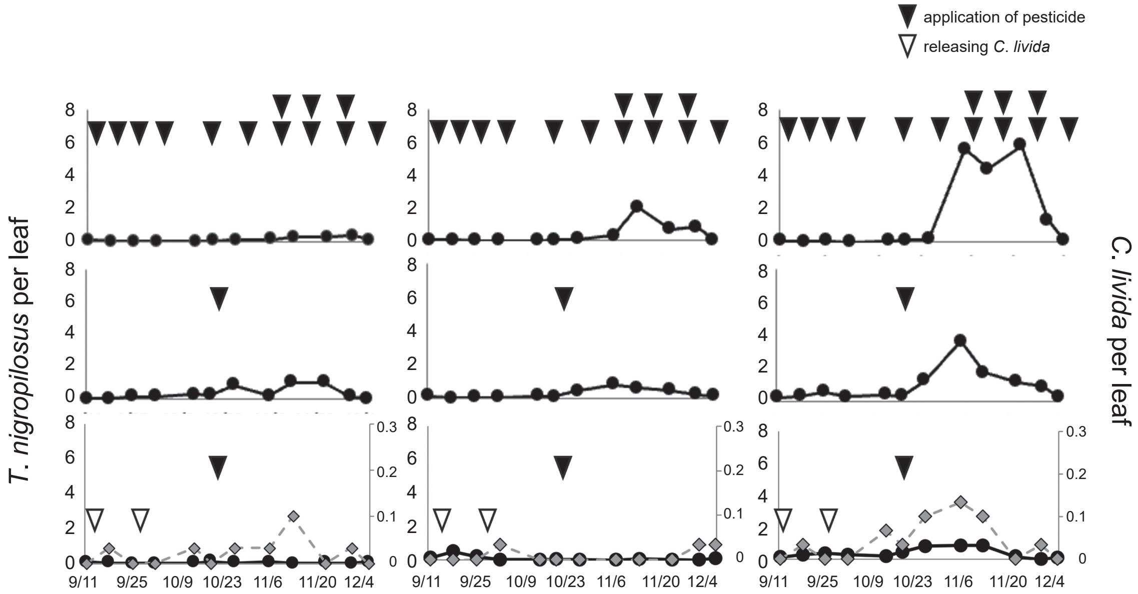

Fig. 1. The numbers of individuals of *T. nigropilosus* (solid black line) and *C. livida* (dashed grey line) per chrysanthemum leaf.

the study period. The abundance in the biological control plots was significantly lower than that in the conventional plots [exp(estimate) = 0.20, 95% CI: 0.08–0.47, z = -3.65, $P < 0.05$]. Despite the regular application of pesticides in the conventional plots (Table 3), there was no significant difference in the abundance of thrips between the conventional and control plots in the counting model [exp(estimate) = 0.58, 95% CI: 0.25–1.34, z = -1.28, $P > 0.05$]. The zero hurdle component (binomial with a logit link) revealed that the control plots was associated with a significant increase in the probability of non-zero observation [exp(estimate) = 10.00, 95% CI: 1.18–84.8, z = 2.11, $P < 0.05$], whereas the biological control plots had no significant effect on this parameter [exp(estimate) = 1.18, 95% CI: 0.38–3.70, z = 0.29, $P > 0.05$], when compared with the conventional plots.

The GLMM indicated that the treatment significantly affected the number of leaves damaged by thrips per plant (type II Wald chi-square test, $\chi^2 = 643.02$, $P < 0.001$). Pairwise comparisons adjusted by Tukey's method revealed that the number of damaged leaves in the biological control plots was significantly lower than in the conventional [GLMM Poisson, exp(estimate) = 0.271, 95% CI: 0.24–0.30, z = -23.69, $P < 0.0001$] and control plots [GLMM Poisson, exp(estimate) = 0.267, 95% CI: 0.24–0.30, z = -24.28, $P < 0.0001$]. There was no significant difference in this variable between the conventional and control plots [GLMM Poisson, exp(estimate) = 0.984, 95% CI: 0.91–1.07, z = -0.47, $P > 0.05$].

We observed some *T. apicalis* on the terminal buds of chrysanthemums after November. In the survey of the quality of products conducted after the harvest, no significant differences were identified among the three treatments in the number of leaves damaged by *T. apicalis* per plant (type II Wald chi-square test, $\chi^2 = 2.463$, $P > 0.05$).

Shapiro-Wilk tests indicated that the data within each treatment deviated significantly from normality (all $P < 0.05$). Therefore, we employed the non-parametric Kruskal-Wallis test. The rate of occurrence of *Tetranychus urticae* (green form) differed significantly among the three treatments, as indicated by the Kruskal-Wallis test ($\chi^2 = 64.15$, $df = 2$, $P < 0.001$). The number of spider mites was significantly higher in the conventional plots

Table 3. History of chrysanthemum and pesticide use in the 2023 *C. livida* release trial.

Date	Crop management	Active ingredient			IRAC code[y]
		Biological control plot	Control plot	Conventional plot[z]	
4 Sep	Transplanting				
12 Sep		*C. livida* [1.0 individuals m^{-2}]		Imidacloprid 20% SC	4A
15 Sep	Pinching				
20 Sep				Abamectin 1.8% EC	6
26 Sep		*C. livida* [1.0 individuals m^{-2}]		Chlorfenapyr 10% SC	13
28 Sep	Pruning				
2 Oct				Prothiofos 45% EC	1B
		Bacillus thuringiensis and the insecticidal proteins it produces	*Bacillus thuringiensis* and the insecticidal proteins it produces		11A
20 Oct				Tebufenpyrad 10% EW	21A
31 Oct				Milbemectin 1.0% EC	6
1 Nov	Turning off supplemental lights				
9 Nov				Cyenopyrafen 30% SC Tolfenpyrad 15% EC	25A 21A
16 Nov				Spinosad 25% WG Acequinocyl 15% SC	5 20B
27 Nov				Sulfoxaflor 9.5% SC Bifenthrin 7.2% SC	4C 3A
1 Dec				Abamectin 1.8% EC	6
14 Dec	Harvesting				
Total number of pesticides		1	1	13	

[z] SC: suspension concentrate; EC: emulsifiable concentrate; EW: emulsion, oil in water; WG: water dispersible granules. See Appendix 2 for the cost per unit, dilution ratio, and applied amount of each pesticide.
[y] IRAC: Insecticide Resistance Action Committee.

than in the biological control and control plots ($P < 0.001$, Wilcoxon rank-sum test). No significant difference in this variable was observed between the biological control and control plots ($P > 0.05$). The occurrence of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae), which is not considered to be economically important, also differed significantly among the three treatments, as shown by the Kruskal-Wallis test ($\chi^2 = 22.33$, $df = 2$, $P < 0.001$). The number of scale insects was significantly lower in the conventional plots than in the biological control and control plots ($P < 0.001$, Wilcoxon rank-sum test). However, there was no significant difference in this variable between the biological control and control plots ($P > 0.05$).

Comparison of net production value

The net production value of each plot is shown in Table 4. As supplemental data, the breakdown of production value by quality, pesticide costs per unit area, and labor costs for pesticide application are provided in Appendices 1, 2, and 3, respectively. The net production value of the conventional plot was 434,430/1,000 m^2, while the control plot was expected to increase revenue by 72,809 yen and the biological control plot by 245,598 yen, compared with the level for the conventional plot.

DISCUSSION

In this study, we explored potential natural

Table 4. Comparison of the net production value per 1,000 m^2 between treatments.

Treatment	Production value (JPY)[z]	Pesticide/biological control agent costs (JPY)[y]	Labor costs for pesticide application (JPY)[x]	Net production value (JPY)
Biological control plot	784,029 (627,223–940,835)	104,004	3,500	680,028 (523,221–836,834)
Control plot	512,160 (409,728–614,592)	4,921	2,625	507,239 (404,807–609,671)
Conventional plot	512,160 (409,728–614,592)	77,730	39,375	434,430 (331,998–536,862)

[z] The production value was estimated based on the unit prices and the quantities. Based on the proportion of damaged leaves in the harvest survey, the quality of chrysanthemums was divided into high grade and medium grade, with unit prices incorporating a ± 20% variation. The quantities were calculated based on the composition of each grade. See Appendix 1 for details.

[y] The costs consist of the unit price, dilution ratio of each pesticide, and amount used per unit area. See Appendix 2 for details.

[x] The hourly rate was set at 1,750 JPN based on the guidelines of OPG (2018). See Appendix 3 for details.

enemies in chrysanthemum fields. As a result, we identified *Campylomma* spp., *G. ochropterus*, *Orius* spp., and *Haplothrips* spp. in chrysanthemum fields on Okinawa Island. Among these, *C. livida*, which has been considered an effective natural enemy of thrips and whitefly (Wang 1994; Qin *et al*. 2004; Kijima *et al*. 2013; Nakaishi 2013), was the most frequent and abundant (Tables 1 and 2).

C. livida is known to prey on up to 482.5 second-instar larvae of *T. palmi* per day (Nakaishi 2013). Since no previous findings of whether it preys on *T. nigropilosus* could be identified, we fed adult and larval thrips in the laboratory to determine whether the *C. livida* adults preyed on them. The results showed that the adult *C. livida* could feed on an average of 9.0 adults or 9.9 second-instar larvae out of 10 *T. nigropilosus* individuals provided within 24 h, suggesting that it can be considered an effective natural enemy of *T. nigropilosus*. Although further study is needed to determine the exact level of predation, it could be close to the amount of predation by *T. palmi* because the body sizes of the thrips and *T. nigropilosus* are comparable.

In the field trials, *C. livida* significantly reduced the number of thrips-damaged plants compared with that in the conventional treatment. The high proportion of zeros likely reflects the patchy distribution of thrips and the effectiveness of treatments in eliminating the pest from many sampling points. Furthermore, the damage to chrysanthemum products was significantly reduced in the biological control plots (Fig. 2). Although the present field study was conducted only single season, these results strongly suggest that the biological control plots are the most effective option for reducing thrips populations and the damage they cause to chrysanthemum products. In conventional chrysanthemum cultivation in Okinawa, pesticides are regularly applied throughout the cultivation period (Fig. 1). Therefore, our surveys for potential natural enemies were limited to the period of May–June, which corresponds to the time when residues have remained in the field for a relatively long time after harvest. However, *C. livida* was established in our field trial from September to December (Fig. 1). As Yasunaga *et al*. (2015) mentioned, this predacious bug is multivoltine, indicating that it has the potential for year-round use for controlling thrips in chrysanthemum. Although there was a concern about it potentially damaging chrysanthemum as *C. livida* has been reported to damage crops (Luo *et al*. 1996), no such damage was observed in this study. These results provide evidence for the effectiveness of *C. livida* for controlling thrips in chrysanthemum.

The findings of this study also suggested that the use of *C. livida* would be associated with a significant increase in profitability, and labor costs would be significantly lower for maintaining the biological control plots than for the conventional plots (Table 4). However,

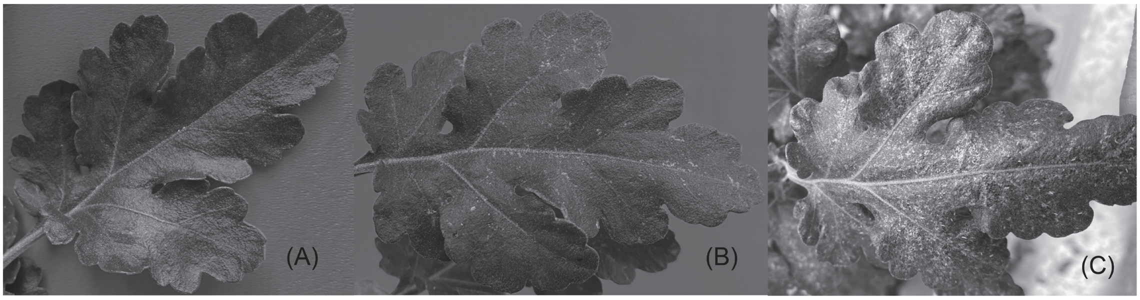

Fig. 2. Examples of leaf damage caused by *T. nigropilosus*. (A) Undamaged leaf. (B) Slightly damaged leaf: visible but minor damage. (C) Heavily damaged leaf: significant damage affecting a large portion of the leaf surface. Leaves classified as "damaged" include both slightly and heavily damaged leaves.

there is a need for more detailed confirmation of the actual profitability relative to the net production value because, despite the fact that unit prices and quality evaluation criteria are significantly influenced by fluctuations in market conditions, we adopted predetermined values for both high- and medium-grade products in our calculation. Moreover, the cost of *C. livida* was calculated based on the price of *O. strigicollis*, assuming that it would also become available on the market. However, since it is the most common indigenous natural enemy found in chrysanthemum, it may be possible to apply it at a lower cost by using insectary plants to attract or retain it.

There was no significant difference between the conventional and control plots in terms of the number of leaves damaged by thrips per plant. The factors contributing to this are unclear, but it is possible that the dense planting of chrysanthemums prevented the chemical solution from reaching the thrips' habitat, as shown by Kijima *et al*. (2014). Our results may indicate that the chemical was not sufficiently effective due to it being applied unevenly. More research is needed to determine whether other factors may have been involved in the lack of a significant difference mentioned above. To prevent the movement of *C. livida* between treatment plots, a variety of pesticides were applied around the netted enclosures at least once a week. However, because the dispersal capacity of *C. livida* is not clearly understood, it remains uncertain whether inter-plot movement was fully prevented. If *C. livida* had moved between plots, such movement would likely have reduced the differences between the natural enemy release plots and the others. Nevertheless, the clear differences detected in this study strongly suggest the effectiveness of releasing *C. livida* as a biological control agent.

Biological control in chrysanthemum cultivation may involve both positive and negative side effects on pests other than thrips. Spider mites, which are known to be the second most important chrysanthemum pest after *T. nigropilosus* (Ganaha-Kikumura *et al*. 2012), were significantly less abundant in the biological and control plots than in the conventional plots. Meanwhile, no significant difference in this regard was observed between the biological and control plots. It was not possible to determine why there was a low level of spider mite infestation in this trial, but the presence of natural enemies of spider mites may have had an effect, as predacious gall midges and predatory mites have been observed on chrysanthemum in other surveys. In other words, as clarified by Bommarco *et al*. (2011) in a study on cabbage, pesticide application may eliminate the natural enemies of spider mites, resulting in a surge in their population. Future research should be performed to clarify this. However, reducing the frequency of pesticide application caused a significantly higher occurrence of a new pest, *P. solenopsis*. Multiple infestations of this scale insect could

cause the wilting of new shoots. Besides, this species is known to be polyphagous (Tanaka & Uesato 2012) and can utilize a variety of weeds, so we should aware of it when reducing pesticide application. There is also an important pest of chrysanthemum, *T. apicalis* (Yasuda 1993). As it belongs to the same family as *C. livida*, it is anticipated that the use of pesticides to control *T. apicalis* may negatively affect *C. livida*. From this point of view, it is thus crucial to develop IPM techniques that do not rely on pesticides.

In this experiment, the density of the release of *C. livida* was set based on that of *O. strigicollis*, and releases were carried out twice- immediately after planting and two weeks later- primarily for convenience. During the trial, the natural enemy was frequently associated with newly emerging shoots (personal observation). This indicates that optimizing the release timing with shoot development may improve establishment. Mass-rearing of this natural enemy is technically feasible, and the availability of commercialized indigenous predatory bugs suggests that its commercialization is also achievable.

It should be noted that using a single natural enemy may induce an enemy-risk effect in pest populations (Culshaw-Maurer *et al*. 2020) and increase the risk of control failure over time due to intraguild predation (Rosenheim 2005). To mitigate or avoid such risks, the development of techniques that enhance the diversity of natural enemies around the fields- such as placing flowering chrysanthemums near field margins- and the use of natural enemies with different ecological niches and functional traits (e.g. activity periods or seasonal occurrences) could contribute to stabilizing pest control. The genus *Ceranisus*, parasitoid wasps identified in this study, known to parasitize multiple thrips species, could be considered as one of the potential candidates.

For large-scale field trials, additional studies will be required, including the assessment of the effects of major insecticides and fungicides, elucidation of developmental traits, determination of optimal release timing and density, evaluation of the necessity of banker plants for conservation of natural enemies and selection of appropriate plant species, as well as the development of countermeasures against emerging pests including scale insects.

Once these aspects are clarified, growers should recognize that the effectiveness of natural enemy releases in chrysanthemum production can vary greatly depending on environmental conditions and crop management practices, which are performed based on their judgment, including the types and timing of pesticide applications.

ACKNOWLEDGMENTS

We sincerely thank Prof. Shiro Nakao (Kyoto Prefectural University) for providing valuable comments on an early draft of this manuscript. We are also grateful to Ms. Kanami Taba and Ms. Chiemi Arasaki (Okinawa Prefectural Plant Protection Center) for their assistance in collecting the field surveys. Special thanks are extended to Dr. Toru Shimizu (Ryukyu Sankei Co. Ltd.) for providing *C. livida* used in the experiments and to Dr. Kazunori Matsuo (Kyushu University) for the identification of parasitoid wasps. Finally, we appreciate the support and assistance provided by all members of Okinawa Prefectural Agricultural Research Center.

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Appendix 1. Breakdown of production value by quality.

Treatment	Quality grade[z]	Unit price (JPY/unit)[y]	Quantity[x]	Production value (JPY)[w]
Biological control plot	High grade	25 (20–30)	20,913	522,825 (418.260–627,390)
	Medium grade	12 (9.6–14.4)	21,767	261,204 (208,963–313,445)
	Subtotal			784,029 (627,223–940,835)
Control plot	High Grade	25 (20–30)	0	0
	Medium Grade	12 (9.6–14.4)	42,680	512,160 (409,728–614,592)
	Subtotal			512,160 (409,728–614,592)
Conventional plot	High Grade	25 (20–30)	0	0
	Medium Grade	12 (9.6–14.4)	42,680	512,160 (409,728–614,592)
	Subtotal			512,160 (409,728–614,592)

[z] A damaged leaf rate of < 20% was considered to represent high grade, while ≥ 20% was considered to represent medium grade. See Fig. 2 for the definition of damaged leaves.

[y] Unit prices for high grade and medium grade were taken with reference to guidelines from OPG (2018). Values in parentheses indicate the range based on a ± 20% variation in unit price.

[x] The total number of chrysanthemums per 1,000 m^2 was set at 42,680 for all treatments. Quantity by grade was calculated according to the percentage of each grade in our harvesting survey.

[w] Values in parentheses indicate the range based on a ± 20% variation.

Appendix 2. Pesticide costs per 1,000 m^2 in Okinawa, Japan.

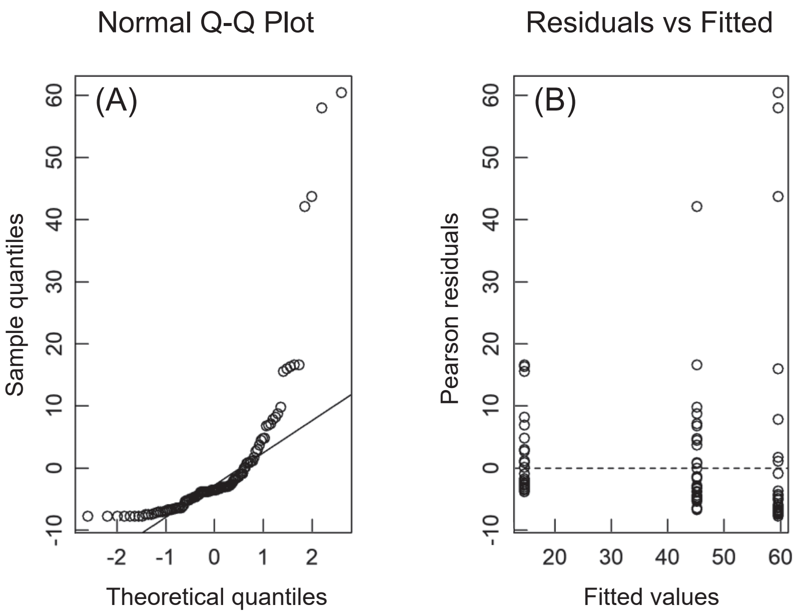
Treatment	Pesticide/biological control agent	Cost per unit (JPY)	Dilution ratio	Applied amount (l)	Quantity used	Unit	Cost per unit area (JPY)
Biological control plot	*C. livida*	49.1[y]	-	-	2,000	individual	98,208
	BT[z]	11.5	1,000	200	200	g	2,296
	Subtotal						104,004
Control plot	*BT*	11.5	1,000	200	200	g	2,296
	Subtotal						2,296
Conventional plot	Imidacloprid	21.0	2,000	150	75	mL	1,574
	Abamectin	9.9	500	450	900	mL	8,952
	Chlorfenapyr	27.4	2,000	150	75	mL	2,058
	Prothiofos	10.0	1,000	200	200	mL	2,004
	Tebufenpyrad	11.1	1,000	200	200	mL	2,224
	Milbemectin	10.2	1,500	200	133	mL	1,362
	Cyenopyrafen	22.6	2,000	300	150	mL	3,395
	Tolfenpyrad	13.1	1,000	300	300	g	3,928
	Spinosad	62.9	5,000	300	60	g	3,775
	Acequinocyl	11.2	1,000	300	300	mL	3,374
	Sulfoxaflor	11.7	1,000	300	300	mL	3,517
	Bifenthrin	29.2	4,000	300	75	mL	2,192
	Subtotal						38,355

[z] *Bacillus thuringiensis* and the insecticidal proteins it produces.

[y] The cost per unit was calculated based on the retail price of *Orius strigicollis* because *C. livida* is not commercially available in Japan.

Appendix 3. Labor costs for pesticide application in Okinawa, Japan.

Treatment	Task	Labor period (h)	Hourly rate (JPY h^{-1})	Total cost (JPY)
Biological control plot	*C. livida*	0.50	1,750	875
	BT	1.50	1,750	2,625
	Subtotal	2.00		3,500
Control plot	*BT*	1.50	1,750	2,625
	Subtotal	1.50		2,625
Conventional plot	Imidacloprid	1.25	1,750	2,188
	Abamectin	3.25	1,750	5,688
	Chlorfenapyr	1.50	1,750	2,625
	Prothiofos	1.50	1,750	2,625
	Tebufenpyrad	1.50	1,750	2,625
	Milbemectin	1.50	1,750	2,625
	Cyenopyrafen	2.00	1,750	3,500
	Tolfenpyrad	2.00	1,750	3,500
	Spinosad	2.00	1,750	3,500
	Acequinocyl	2.00	1,750	3,500
	Sulfoxaflor	2.00	1,750	3,500
	Bifenthrin	2.00	1,750	3,500
	Subtotal	22.50		39,375



Supplementary Fig. 1. Residual diagnostic plots for the count component of the hurdle model. (A) Quantile-quantile (Q-Q) plot of Pearson residuals shows no major deviation from normality. (B) The residuals versus fitted values plot indicates homoscedasticTity and no apparent pattern, suggesting an adequate model fit.

利用本土天敵進行沖繩菊花田害蟲永續管理的挑戰

Tomoko Ganaha-Kikumura[1,*] and Atsushi Honma[2,3,4]

摘要

Ganaha-Kikumura, T. and A. Honma. 2026. Challenges for sustainable pest management utilizing indigenous natural enemies in chrysanthemum fields in Okinawa. J. Taiwan Agric. Res. 75(1):117–134.

菊褐斑薊馬 (*Thrips nigropilosus*) 是沖繩所產菊花最重要的經濟害蟲。為了管理該物種，需進行害蟲整合管理，包括在菊花上使用天敵，以作為友善且永續的害蟲防治方法。為開發以本土天敵防治此菊花害蟲的技術，遂進行下列研究：(1) 在菊花田中調查菊褐斑薊馬的天敵； (2) 評估潛在天敵中華微刺盲椿象 (*Campylomma livida*) 對菊褐斑薊馬的捕食能力；(3) 以田間試驗評估增強釋放 *C. livida* 的有效性。已鑑定天敵包含 *Campylomma* spp.、*Geocoris ochropterus*、*Orius* spp.、*Haplothrips* spp. 以及 *Ceranisus* sp.，其中以 *C. livida* 的數量最多。在實驗室中，每隻 *C. livida* 成蟲於 24 小時內，可取食菊褐斑薊馬之成蟲與若蟲，分別為 9 隻與 9.9 隻。因此，我們利用中華微刺盲椿象進行實地試驗，以檢查其降低菊褐斑薊馬密度與減少損害的效果，並與定期噴灑農藥的傳統樣區或未噴灑農藥的對照樣區進行比較，結果顯示在生物防治樣區 (釋放了 *C. livida*) 所受薊馬危害的植物數量減少 1/5，菊花受損程度亦降低至 1/3 以下，顯示中華微刺盲椿象可在菊花田有效防治菊褐斑薊馬。

關鍵詞：菊褐斑薊馬、琉球、生物防治、中華微刺盲椿象。

投稿日期：2025 年 5 月 7 日；接受日期：2025 年 11 月 19 日。

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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):135–146 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0008

研究報告

草莓「台農 1 號」及其親本園藝性狀與抗白粉病之研究

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摘要

蕭翌柱、林宗俊、李裕娟。2026。草莓「台農 1 號」及其親本園藝性狀與抗白粉病之研究。台灣農業研究 75(1):135–146。

草莓「台農 1 號」係由「香水」(母本) 與「桃園 1 號」(父本) 雜交授粉所育成的品種。本研究目的在調查「台農 1 號」與二親本的園藝性狀並檢測其抗白粉病能力。結果顯示，在本試驗條件下之「台農 1 號」(18.5 cm) 與「香水」(18.6 cm) 植株顯著高於「桃園 1 號」(14.8 cm)；植株寬度則是「桃園 1 號」(39.3 cm) 大於「台農 1 號」(37.5 cm) 與「香水」(36.8 cm)。「桃園 1 號」單株萌生的匍匐蔓數 (7.6 條)、中間小葉葉長 (10.6 cm)、葉寬 (10.2 cm) 及三出複葉的葉面積 (308.6 cm^2)，均顯著大於其他 2 個品種，但葉柄長度 (11.0 cm) 則最短。「香水」單季單株平均總花序數 (7.1 支)、單一花序收穫果實數 (12.7 顆) 及果實平均鮮重 (13.2 g) 均高於「台農 1 號」與「桃園 1 號」；不過，「台農 1 號」果實硬度 (0.8–0.9 kg cm^{-2}) 較高，且可溶性固形物含量 (9.5–10.4°Brix) 與「桃園 1 號」(8.5–11.8°Brix) 相近，「香水」(6.2–7.4°Brix) 則偏低。「桃園 1 號」果實酸度 0.34–0.44% 比其他 2 個品種高，抗白粉病能力則不如「香水」與「台農 1 號」，上述各項試驗結果可提供農友們作為栽培草莓之參考。

關鍵詞：抗病性、草莓「台農 1 號」、草莓「桃園 1 號」、草莓「香水」、白粉病。

前言

草莓在分類學上歸類為薔薇科 (Rosaceae) 草莓屬 (*Fragaria*) 多年生草本植物，原生於南美洲、歐洲及東亞等區域，若以草莓育種研究歷史而言，*Fragaria* ×*ananassa* Duch. 應該是最重要的八倍體種間雜交種，即體細胞中含有 8 個染色體組，共計 56 條染色體 (Hancock *et al*. 2008)。該品種最初是在 18 世紀中期時，由被攜帶到法國進行培育的智利 *F. chiloensis* L. P. Mill. 與來自美國東岸的 *F. virginiana* Duch. 兩個原生種，在偶然情形下自然雜交授粉與結果，實生苗經栽培後收穫之果實，因具有類似於鳳梨的香氣，最終遂由該國植物學家 A. N. Duchesne 鑑定及命名；至今，世界許多知名的草莓品種，仍可溯源為此一種間雜交種之雜交後代 (Karim *et al*. 2015)。

在自然界中，草莓花朵由蜜蜂等昆蟲或風媒授粉後，花托部位會膨大發育成聚合果，其含有礦物質、維生素 C、醣類及果膠，以及對人體有益之鞣花酸與阿魏酸等多酚化合物，以及花青素、兒茶素等黃酮類抗氧化成分 (Giampieri *et al*. 2014; Mancini *et al*. 2023)，不但風味酸甜可口且兼具香氣，廣受世界各地消費者的喜愛。2023 年聯合國糧食及農業組織數據資料庫 (https://www.fao.org/faostat/en/#data/QCL) 統計資料顯示，全球現今約有 78 個國家商業量產草莓，全年總收穫面積達 434,493 ha，其中栽培面積最大的前 5 個國家，依序為中國 155,750 ha (產量 27,025.8 kg ha^{-1})、俄羅斯 39,690 ha (產量 6,580.1 kg ha^{-1})、波蘭 30,000 ha (產量 6,483.3 kg ha^{-1})、美國 22,986 ha (產量 54,385.3 kg ha^{-1})，以及土耳其 21,984 ha (產量 30,786.8 kg ha^{-1})。我國農業部在 2025 年

投稿日期：2025 年 9 月 12 日；接受日期：2025 年 11 月 25 日。
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發布的《農業統計年報》(https://agrstat.moa.gov.tw/sdweb/public/book/Book.aspx) 資料也記載，2024 年的國內草莓總收穫面積為 598 ha，年產量 12,395 kg ha^{-1}，其中以苗栗縣栽培面積 537 ha 最大，其他依序為臺中市 (15 ha)、南投縣 (10 ha) 及新竹縣 (7 ha)。

苗栗縣大湖鄉迄今仍是國內最主要的草莓量產區域，但因近年來全球氣候暖化日益嚴重，加上秋、冬兩季平均氣溫持續偏高，且受致災性暴雨等極端天氣影響下，當地農友只得選種較耐熱與高產的「香水」品種，或由國內業者自行選拔推廣的「蘋果」與「美姬」等品種(系)；另有農企業從國外引入「紅顏」、「佐賀清香」或白草莓「桃薰」、「雪兔」及「天使」等品種在溫室設施內栽培；至於，三十多年前由國人自日本引進知名「豐香」種苗，再分贈給農業部桃園區農業改良場進行無性繁殖與區域試驗，最終選拔與命名的優良品種「桃園 1 號」(Council of Agriculture 2011)，因近年感染嚴重的炭疽病或白粉病等病害，並遭受二點葉蟎、臺灣花薊馬及蚜蟲侵擾，加上開花期受氣候暖化與高溫影響而延遲，導致整體果實品質與產量降低，故目前商業栽培面積已急遽縮減 (Chien *et al*. 2021)。為促進我國農村經濟與產業的發展並增加農民收益，桃園區農業改良場在多年前已選育出「桃園 4 號 - 紅冠」品種並推廣種植 (Luo *et al*. 2012)；農業部苗栗區農業改良場也出版《草莓病蟲害預防性管理手冊》輔導農友進行病蟲防治，並介紹有大果與高甜度等特性的「苗栗 1 號 - 戀香」(Chung *et al*. 2020)；農業部農業試驗所 (農試所) 也發表歷經 10 年選育且具備醇厚香氣與口感良好的「台農 1 號」優良品種。

草莓「台農 1 號」係由植株強健的「香水」(母本) 與果香濃郁的「桃園 1 號」(父本) 雜交授粉取得實生苗，再經多年品系選拔與比較試驗後所育成的品種。Chen & Shiau (2020) 發表的研究報告顯示，應用日本開發的草莓品種鑑定 cleaved amplified polymorphic sequence (CAPS) 標誌中之 *APX* 基因座，可明確鑑別「台農 1 號」(品系代號：「SF4– 初」) 與父、母本的親緣關係。目前，此一品種雖已技術授權國內 2 家大型農企業進行種苗繁殖及推廣栽培，但農友們在購買苗株時，常會詢問「台農 1 號」與二親本的植株性狀差異、果實風味異同及抗病性強弱等問題。因此，本研究旨在調查比較此 3 個品種的園藝性狀，並檢測果實鮮重、硬度、可溶性固形物含量 (soluble solid content; SSC)、酸度及抗白粉病能力之差異，以提供農友們多一項參考資料與栽培選擇，進而量產品質優良的草莓並造福廣大消費群眾。

材料與方法

供試材料來源

本試驗使用的草莓「台農 1 號」與「香水」(母本) 及「桃園 1 號」(父本) 二個親本 2.5 吋盆苗，係由農試所遺傳生技組與轉譯中心於 2023 年 6–9 月，在 18-7 溫室設施內進行營養繁殖，並於 10 月起選取供試品種生育健壯的苗株各 30 株，將其定植於添加泥炭土與珍珠石無土介質 (Gramoflor GmbH Co. KG, Neuenkirchen-Vörden, Germany) 之 7 吋塑膠盆，置於自然光照條件且設有降溫水牆之 12-1 溫室內培育，試驗期間使用硝磷基黑旺特 5 號有機質複合肥料 (N : P_2O_5 : K_2O = 16 : 8 : 12) (台灣肥料股份有限公司，臺灣臺北市) 作為基肥，定植後 1 月，每株施用 3 g 好康多 1 號緩效性肥料 (N : P_2O_5 : K_2O = 14 : 12 : 13) (台灣傑康農業科技股份有限公司，臺灣臺北市) 作為追肥。

「桃園 1 號」、「台農 1 號」及「香水」植株園藝性狀、花序數及果實質量調查

參照農業部 2022 年公告的《草莓品種試驗檢定方法》、《草莓品種性狀表》及《草莓品種性狀調查表圖例說明》(https://law.moa.gov.tw/LawContent.aspx?id=GL001486)，在如下述規定的生育階段，進行草莓「桃園 1 號」、「台農 1 號」及「香水」植株之園藝性狀調查與比較工作。(1) 在生育結束後之育苗期間 (約 6–9 月) 調查匍匐莖數目；(2) 在果實成熟期前 (約在苗株定植後 2.5 月)，調查成熟植株的株

高 (地上部的基部到頂端之距離)、株寬 (冠部葉片向外側伸展兩端最大直線距離)、葉柄長、頂生小葉葉長、葉寬及三出複葉之葉面積；(3) 在 2023 年 11 月中旬至隔年 4 月底止，草莓開花結果期間 (合計 4 期花)，調查供試品種單季單株平均花序數、每一花序收穫商品化以上 (6 g/顆) 果實數及計算單一果實平均重量；此外，將收穫的果實依重量分成 4 個等級，即第 1 級果 (> 20 g)、第 2 級果 (15–20 g)、第 3 級果 (10–15 g) 及第 4 級果 (< 10 g)。各等級單一果實分別以電子天秤與游標尺測量鮮重、果長及果肩寬，再依序使用硬度計 (FT–327, Mc CORMICK, Fabbrico, Italia) 檢測果實硬度 (kg cm^{-2})；檢測硬度後之果實則壓榨取汁，以微量吸管吸取 0.1 mL 果汁滴在糖度計 (MASTER-M, ATAGO CO., LTD., Tokyo, Japan) 玻璃基板檢測其 SSC (°Brix)；另以微量吸管吸取 0.2 mL 果汁，注入數位酸度計 (PAL–BX/ACID 4, ATAGO CO., LTD., Tokyo, Japan) 感應槽，再加入 9.8 mL 去離子水攪拌稀釋後，測定酸度 (%) 並取至數字小數點後 2 位。

「桃園 1 號」、「台農 1 號」及「香水」抗白粉病能力檢測

菌種來源

本試驗使用的白粉病菌株，係採集自苗栗縣大湖地區罹患白粉病之草莓植株。有關此一白粉病菌種的核醣體去氧核醣核酸 (ribosomal deoxyribonucleic acid; rDNA) 定序與菌種鑑定，係參考學者們報導使用的引子與聚合酶連鎖反應 (polymerase chain reaction; PCR) 擴增 rDNA 內轉錄區間 (internal transcribed spacer; ITS) 的方法 (Takamatsu *et al*. 1998; Ito & Takamatsu 2010)。試驗以自然感染白粉病葉片上孢子為材料，使用無菌牙籤挑取置於 1.5 mL 微量離心管中，採微波破菌法破壞細胞壁後，以 TE 緩衝液回溶作為粗萃 DNA 模板。以 ITS4/ITS5 引子擴增約 550 bp 片段後進行定序，透過美國國家生物技術信息中心 (National Center for Biotechnology Information; NCBI) 基本局部對齊工具 (basic local alignment search tool; BLAST) 比對結果與 *Podosphaera aphanis* (舊名：*Sphaerotheca macularis* f. sp. *fragariae*) 相似度達 99%，故鑑別此一白粉病菌株為 *Podosphaera aphanis*。

菌體增殖及接種

選取具有 4–5 片展開葉之草莓「桃園 1 號」與「紅顏」二個品種的健康植株為宿主，進行葉面人工接種，先以自然感染白粉病之罹病葉片摩擦供試植株之葉面後，再置入適合白粉病菌孢子萌發的植物生長箱進行栽培與觀察，設定的環境溫度為 24–26℃、相對濕度 (relative humidity; RH) 60−70% 及光照 12 時。當葉片出現如 Sargent *et al*. (2019) 描述之白色斑塊與粉狀菌絲層表徵時 (約接種後 10–14 日)，即可判定其為白粉病罹病葉片且菌株的分生孢子已附著並開始萌發；此時，使用含有 0.05% (v/v) Tween 80 之無菌水溶液，清洗附著大量孢子的葉片表面，並收集這些含病原菌分生孢子的清洗液作為接種用懸浮液，調配濃度為 3 × 10^4 spores mL^{-1}，每一供試植株葉片表面均勻噴灑至有水珠滴落，再置入植物生長箱 4 週後調查葉片罹病率。有關葉片白粉病罹病率依下列公式計算：罹病率 (%) = (罹病葉片數/總葉片數) × 100。

試驗統計分析

以上試驗採完全逢機設計，各供試品種以 7 吋盆栽種單一健壯苗株，計 3 重複，每 1 重複 10 株，總數為 30 株。園藝性狀調查數據，以 CoStat 6.311 軟體進行變方分析 (analysis of variance; ANOVA)，在 5% 顯著性水準下以最小顯著差異性測驗 (least significant difference test; LSD test) 檢定各品種間差異的顯著性。單季單株花序總數等調查項目與取得的各級果實檢測樣品數為 30 株 (個)，獲得的資料則計算其算術平均值與標準差 (standard deviation; SD)。各供試草莓品種抗白粉病能力試驗也為 3 重複，每 1 重複使用 2.5 吋盆苗各 10 株，所得數據資料以 CoStat 6.311 統計軟體進行變方

分析，在 5% 顯著性水準下以最小顯著差異性測驗檢定各處理間差異的顯著性。

結果

「桃園 1 號」、「台農 1 號」及「香水」植株園藝性狀比較

調查草莓「台農 1 號」與其親本 –「桃園 1 號」(父本) 及「香水」(母本) 供試植株各項園藝性狀顯示 (表 1、圖 1)，「台農 1 號」與「香水」植株平均高度分別為 18.5 與 18.6 cm，二者比較後無顯著差異，但皆顯著高於「桃園 1 號」(14.8 cm)；植株平均寬度則是「桃園 1 號」(39.3 cm) 顯著大於「台農 1 號」(37.5 cm) 與「香水」(36.8 cm)。統計單季單株平均萌生的匍匐蔓數量，以「桃園 1 號」最高為 7.6 條，「台農 1 號」次之 (6.9 條)，「香水」萌生的匍匐蔓數量在夏季育苗期間較少，平均僅有 5.7 條，三者經比較後皆有顯著差異。「台農 1 號」與「香水」葉柄平均長度分別為 12.0 與 11.9 cm，二者比較後無顯著差異，但皆顯著長於「桃園 1 號」之 11.0 cm；若測量比較各供試品種中間小葉的平均葉長、葉寬，以及三出複葉的葉面積，則以「桃園 1 號」最高，分別為 10.6 cm、10.2 cm 及 308.6 cm^2，且均顯著高於「台農 1 號」(9.4 cm、8.8 cm、241.2 cm^2) 與「香水」(9.1 cm、8.6 cm、229.5 cm^2)。

「桃園 1 號」、「台農 1 號」及「香水」花序數與果實質量調查

表 2 結果顯示，在當年度 12 月至隔年 4 月底止，調查各供試品種單季合計 4 個花期之單株平均總花序數，結果以「香水」的 7.1 ± 1.9 支較高，「台農 1 號」與「桃園 1 號」分別為 6.4

表 1.　草莓「桃園 1 號」(父本)、雜交後裔「台農 1 號」及「香水」(母本) 植株園藝性狀之比較。

Table 1.　Comparison of plant horticultural characteristics of strawberry 'Taoyuan No. 1' (male parent), its hybrid offspring 'Tainung No. 1', and 'Xiang-Shui' (female parent).

Variety	Plant height (cm)	Plant width (cm)	No. of stolon	Petiole length (cm)	Terminal leaflet leaf length (cm)	Terminal leaflet leaf width (cm)	Trifoliolate leaf area[y] (cm^2)
'Taoyuan No. 1'	14.8 b[z]	39.3 a	7.6 a	11.0 b	10.6 a	10.2 a	300.8 a
'Tainung No. 1'	18.5 a	37.5 b	6.9 b	12.0 a	9.4 b	8.8 b	241.2 b
'Xiang-Shui'	18.6 a	36.8 b	5.7 c	11.9 a	9.1 b	8.6 b	229.5 b

[z] Means within each column followed by the same letter are not significantly different at 5% level by Fisher's protected least significance difference (LSD) test (n = 3).
[y] Refer to the legend of the strawberry variety characteristics survey table.

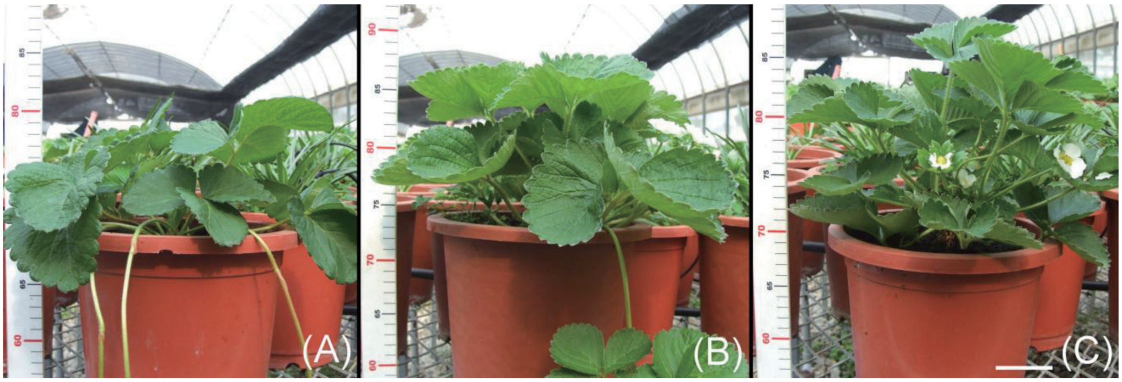

圖 1.　草莓 (A)「桃園 1 號」(父本)、(B) 雜交後裔「台農 1 號」及 (C)「香水」(母本) 植株之外觀型態。

Fig. 1.　The appearance of strawberry (A) 'Taoyuan No. 1' (male parent), (B) its hybrid offspring 'Tainung No. 1', and (C) 'Xiang-Shui' (female parent). (Scale bar = 5 cm).

表 2. 草莓「桃園 1 號」(父本)、雜交後裔「台農 1 號」及「香水」(母本) 單季單株花序總數、每花序收穫果實數及單一果實平均重量之比較。

Table 2. Comparison of total No. of inflorescences in a single season, No. of fruits harvested per inflorescence, and the average weight of a single fruit per plant of strawberry 'Taoyuan No. 1' (male parent), its hybrid offspring 'Tainung No. 1', and 'Xiang-Shui' (female parent).

Variety	No. of inflorescences in a single season	No. of fruits harvested per inflorescence	Mean weight per single fruit (g)
'Taoyuan No. 1'	6.3 ± 0.3[z]	11.8 ± 0.9	11.9 ± 4.2
'Tainung No. 1'	6.4 ± 1.7	12.3 ± 0.9	12.0 ± 5.2
'Xiang-Shui'	7.1 ± 1.9	12.7 ± 1.1	13.2 ± 4.3

[z] Means in the same column are indicate arithmetic mean ± standard deviation (*SD*).

± 1.7 與 6.3 ± 0.3 支較低。調查各品種單一花序採收到的果實數，並將全數收穫的果實稱重記錄後平均之，則「香水」單一花序平均可收穫 12.7 ± 1.1 顆果實，且收穫的果實平均鮮重為 13.2 ± 4.3 g，此二數值均相對高於「台農 1 號」(12.3 ± 0.9 顆、12.0 ± 5.2 g) 與「桃園 1 號」(11.8 ± 0.9 顆、11.9 ± 4.2 g)。前述表 2 各項數值雖有高低之別，但在統計上無顯著差異。

表 3 結果顯示，將各供試品種單季收穫的果實依重量分成 4 個等級 (> 20 g、15–20 g、10–15 g 及 < 10 g) 再分別進行質量性狀檢測記錄，則「香水」果實第 1–4 級的平均重量，分別為 26.2 ± 3.1、17.5 ± 0.9、13.6 ± 2.0 及 9.0 ± 0.4 g，略高於「台農 1 號」之 25.9 ± 3.1、17.4 ± 1.4、12.2 ± 1.5 及 8.1 ± 2.3 g，以及「桃園 1 號」的 24.0 ± 2.2、16.7 ± 0.6、12.3 ± 1.5 及 9.0 ± 0.8 g。利用游標尺量測供試品種各等級果實的平均果肩寬與果長時，若以鮮重 20 g 以上之第 1 級果為例，仍是以「香水」的 4.0 ± 0.3 與 4.6 ± 0.6 cm 較高，「台農 1 號」為 3.9 ± 0.4 與 4.5 ± 0.5 cm，至於「桃園 1 號」則是 3.9 ± 0.2 與 4.7 ± 0.5 cm，綜觀 3 個供試品種的果形大多呈現圓錐或短圓椎形 (圖 2)。檢測 3 個供試品種各級果實的硬度則顯示，「台農 1 號」均較其他二品種硬實，其果實硬度介於 0.8 ± 0.1–0.9 ± 0.2 kg cm^{-2}，「桃園 1 號」為 0.6 ± 0.2–0.7 ± 0.3 kg cm^{-2}，「香水」則是 0.6 ± 0.1–0.7 ± 0.2 kg cm^{-2}。檢測各供試品種 4 個等級果實的 SSC 可知，「桃園 1 號」介於 8.5 ± 1.2–11.8 ± 1.3°Brix，「台農 1 號」為 9.5 ± 1.8–10.4 ± 2.8°Brix，「香水」果實的 SSC 則相較偏低，僅有 6.2 ± 0.3–7.4 ± 1.6°Brix，這些數值也表示此一品種甜度較低。若檢測各供試品種果實的酸度，則以「桃園 1 號」酸度較高為 0.34 ± 0.07–0.44 ± 0.12%，「台農 1 號」為 0.28 ± 0.08–0.36 ± 0.14%，「香水」酸度為 0.27 ± 0.07–0.31 ± 0.07%。前述表 3 各項數值雖有高低之別，但在統計上無顯著差異。

「桃園 1 號」、「台農 1 號」及「香水」抗白粉病能力檢測

本試驗以供試草莓品種之全株葉片數與感染白粉病之葉片數進行調查，並依比例計算罹病率，結果顯示「香水」品種在整個試驗觀察期間內，所有葉片皆未發現白粉病的徵狀，表面保持完整且無白色斑點，經計算白粉病的罹病率為 0% (圖 3)，顯示運用植物生長箱控制適合白粉病菌株孢子萌芽的溫、溼度環境時，「香水」全植株仍未受到病原菌侵襲。在相同條件下之「桃園 1 號」品種部分葉片則可見明顯的病斑，甚至出現白色菌絲與粉狀物嚴重覆蓋之情形，經統計罹病葉片占全葉片比例為 22.3%，罹病率顯著高於其他二個品種；相較之下，「台農 1 號」植株雖也有少量病徵出現，但分布範圍較小，僅見少數葉片出現些微白粉病斑，計算整體罹病葉片比例僅為 4.5%，若與「香水」比較並無顯著差異，但顯著低於「桃園 1 號」的白粉病罹病率。

討論

在傳統草莓育種的流程中，多先挑選植株性狀表現優異的親本進行種間雜交授粉，再經長期田間或設施內試種觀察與比較後，自眾多

表 3. 草莓「桃園 1 號」(父本)、雜交後裔「台農 1 號」及「香水」(母本) 果實質量性狀之比較。

Table 3. Comparison of fruit quality properties of strawberry 'Taoyuan No. 1' (male parent), its hybrid offspring 'Tainung No. 1', and 'Xiang-Shui' (female parent).

Variety	Size	Fresh weight (g)	Shoulder width (cm)	Length (cm)	Hardness (kg cm^{-2})	Soluble solids content (°Brix)	Acidity (%)
'Taoyuan No. 1'	More than 20 g	24.0 ± 2.2[z]	3.9 ± 0.2	4.7 ± 0.5	0.7 ± 0.1	8.5 ± 1.2	0.39 ± 0.07
	15–20 g	16.7 ± 0.6	3.2 ± 0.2	4.0 ± 0.3	0.7 ± 0.1	11.8 ± 1.3	0.44 ± 0.12
	10–15 g	12.3 ± 1.5	3.1 ± 0.2	3.8 ± 0.4	0.6 ± 0.2	9.2 ± 0.9	0.36 ± 0.09
	Less than10 g	9.0 ± 0.8	2.6 ± 0.2	3.5 ± 0.3	0.7 ± 0.3	8.8 ± 1.3	0.34 ± 0.07
'Tainung No. 1'	More than 20 g	25.9 ± 3.1	3.9 ± 0.4	4.5 ± 0.5	0.8 ± 0.1	9.5 ± 1.8	0.28 ± 0.08
	15–20 g	17.4 ± 1.4	3.5 ± 0.3	4.0 ± 0.4	0.8 ± 0.2	9.8 ± 1.4	0.31 ± 0.09
	10–15 g	12.2 ± 1.5	3.1 ± 0.2	3.5 ± 0.3	0.9 ± 0.2	9.6 ± 1.5	0.34 ± 0.09
	Less than10 g	8.1 ± 2.3	2.5 ± 0.3	3.0 ± 0.5	0.9 ± 0.2	10.4 ± 2.8	0.36 ± 0.14
'Xiang-Shui'	More than 20 g	26.2 ± 3.1	4.0 ± 0.3	4.6 ± 0.6	0.7 ± 0.2	6.7 ± 0.9	0.27 ± 0.07
	15–20 g	17.5 ± 0.9	3.5 ± 0.1	3.9 ± 0.3	0.7 ± 0.1	7.0 ± 1.0	0.27 ± 0.08
	10–15 g	13.6 ± 2.0	3.3 ± 0.2	3.6 ± 0.2	0.6 ± 0.2	7.4 ± 1.6	0.31 ± 0.07
	Less than10 g	9.0 ± 0.4	2.9 ± 0.1	3.0 ± 0.2	0.6 ± 0.1	6.2 ± 0.3	0.30 ± 0.02

[z] Means in the same column indicate arithmetic mean ± standard deviation (*SD*).

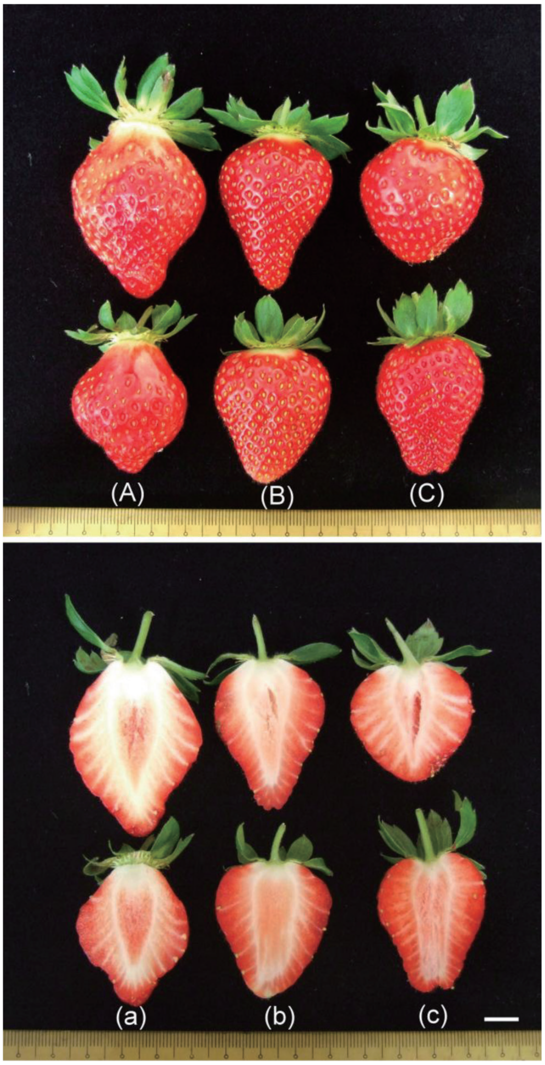

圖 2. 草莓 (A、a)「桃園 1 號」(父本)、(B、b) 雜交後裔「台農 1 號」及 (C、c)「香水」(母本) 果實外觀及其縱剖面之型態。

Fig. 2. The appearance and longitudinal cross–section of strawberry (A, a) ‘Taoyuan No. 1’ (male parent), (B, b) its hybrid offspring ‘Tainung No. 1’, and (C, c) ‘Xiang-Shui’ (female parent). (Scale bar = 1 cm)

的實生苗中，選拔出大果、高產、鮮食風味良好或兼具抗、耐病的優良品系 (Hancock *et al*. 2008)。《草莓品種試驗檢定方法》與《草莓品種性狀表》制訂的數十個植株性狀調查項目，皆是以「桃園 1 號」作為對照品種，根據學者們研究指出，以草莓「桃園 1 號」作為對照組進行栽培試驗時，該品種植株多呈開展狀，高度約 13.4 cm、寬度可達 38.2 cm (Lee *et al*. 1993; Luo *et al*. 2012)，此與本試驗所取得的數據與外觀影像相似 (表 1、圖 1)，亦即植株呈現開張型的「桃園 1 號」平均株高 14.8 cm，顯著比半直立型的「香水」(18.6 cm) 與「台農 1 號」(18.5 cm) 矮，但植株寬幅 (39.3 cm) 顯著大於「香水」(36.8 cm) 與「台農 1 號」(37.5 cm)；另外，「桃園 1 號」葉柄平均長度約 11.0 cm，顯著比「香水」(11.9 cm) 與「台農 1 號」(12.0 cm) 短，但測量中間小葉之葉幅並估算三出複葉的葉面積，則是「桃園 1 號」顯著大於「香水」與「台農 1 號」；前述資料也與早年「桃園 1 號」育種者描述的植株性狀相符合，亦即該品種株型呈披散狀，大葉、質硬、葉色濃綠及葉柄與花梗偏短 (Council of Agriculture 2011)。

《草莓品種性狀表》載明調查各供試品種匍匐蔓數量時，應於每年春季生育期結束後之育苗期間為之 (約 6–9 月)，且作為對照品種的「桃園 1 號」萌生匍匐蔓數位列中等數量 (6–15 條)。表 1 試驗結果也顯示「桃園 1 號」平均匍匐蔓萌生數目為 7.6 條，與前述性狀調查表所列對照品種之標準相符合；「台農 1 號」平均匍匐蔓萌生數為 6.9 條，雖低於「桃園 1 號」(父本)，但仍在中等標準範圍內；至於，「香水」(母本) 平均匍匐蔓萌生數量僅為 5.7 條，雖顯著低於前二者，然實際應用草莓二段式母本繁殖系統進行栽培時，「香水」匍匐蔓的營養繁殖倍率並不亞於「桃園 1 號」(Chang & Chung 2021; Chung *et al*. 2021)，其原因可能是不同品種間生育習性的差異，換言之，適合「香水」匍匐蔓大量萌生的時期，可能比「桃園 1 號」與「台農 1 號」稍晚，因此，當本試驗在夏季高溫育苗期間進行調查時，「香水」萌生的匍匐蔓數才會低於前二者。

有關存在於草莓不同品種間遺傳質多樣性的差異，可由下列幾項園藝性狀與生育習性之表現加以區分，例如，果實風味、香氣、植株耐熱與耐寒及抗病蟲害等能力，以及其他與產量相關的生理性狀，包括開花周期數、匍匐蔓數、果實大小或花誘導期的長度等 (Hancock *et al*. 2001)。多項遺傳學研究發現，草莓果實大小是屬於親本數量遺傳，由 6–8 對等位基因

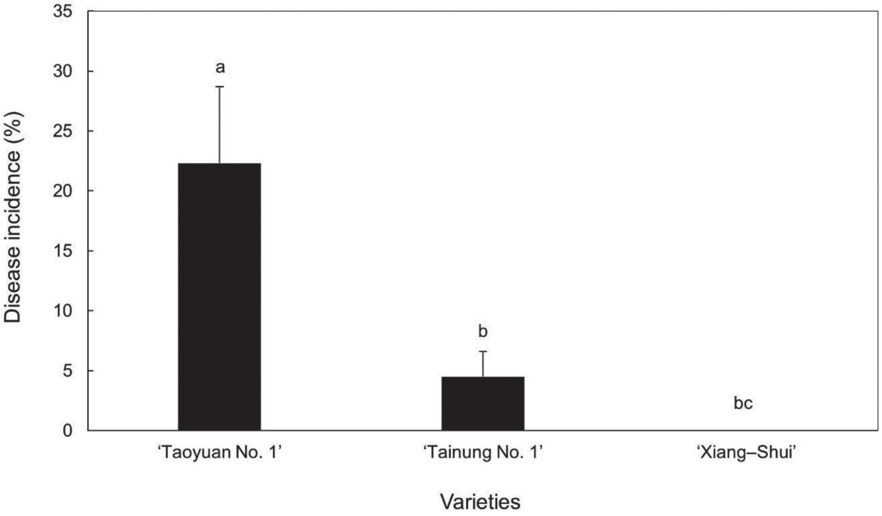

圖 3.　草莓「桃園 1 號」(父本)、雜交後裔「台農 1 號」及「香水」(母本) 葉片之白粉病罹病率。

Fig. 3.　Disease incidence of powdery mildew on leaves of strawberry 'Taoyuan No. 1' (male parent), its hybrid offspring 'Tainung No. 1', and 'Xiang-Shui' (female parent). Vertical bars indicate standard deviation (n = 3). Mean values with the same letter are not significantly different at 5% level by Fisher's protected least significance difference (LSD) test (n = 3).

控制果實的膨大，且因草莓為聚繖花序，開花結果時從總花梗中心主支到分支，果實尺寸會由最大果依序遞減，惟不同基因型間之減少程度仍有差異 (Hancock *et al*. 2008)。本試驗自 10 月初進行草莓苗定植，11 月中旬至 12 月左右成熟株依次開花結果直至隔年 4 月底止，在這此期間約可收穫四期花，亦即每一期花序從始花到果實陸續成熟採收的時間可持續 1 個多月。因此，統計「香水」單季單株萌生的總花序數平均 7.1 ± 1.9 支，且單一花序平均可收穫達商品化標準以上 (6 g/顆) 之果實 12.7 ± 1.1 顆 (表 2)，此數值比「桃園 1 號」(6.3 ± 0.3 支、11.8 ± 0.9 顆/支) 與「台農 1 號」(6.4 ± 1.7 支、12.3 ± 0.9 顆/支) 高，主要原因可能也與品種間之遺傳質差異有關，亦即「香水」開花習性除一般聚繖型花序外，也比「桃園 1 號」更容易從總花梗旁萌生單梗花朵，經授粉後呈單梗結果型態的果實，因植株養分分配較為集中，故生成大果的比率較多，鮮果重量相對也較高。

影響整體草莓果實大小、產量多寡，以及品質高低的因子，除需考量前述不同品種間遺傳質之差異外，也要留意栽種地區的天候、氣溫等環境條件 (Islam *et al*. 2021)，以及栽培期間的肥分管理，例如，設施高架栽植的「桃園 1 號」草莓苗，在每一植株給予 N : P_2O_5 : K_2O 比例為 2.6 : 2.2 : 2.6 g 的肥料量下，經定植 60 日後可得到較高的乾物重與產量，且平均單果鮮重為 10.5 g、果實長度 3.7 cm、果實寬度 2.8 cm 及 SSC 為 8.9°Brix (Chang *et al*. 2007)。本研究雖未進行肥料用量試驗，但在定植與栽培期間適度施用基肥與緩效性肥料，收穫的「桃園 1 號」整體果實平均鮮重仍有 11.9 ± 4.2 g，且 SSC 達到 8.5–11.8°Brix (表 2、表 3)，顯示果實品質堪稱良好。SSC 與酸度是評估草莓口感、成熟度及採收時間的關鍵參數，通常也適用於劃分品質等級 (Weng *et al*. 2020)。ElMasry *et al*. (2007) 認為水果品質是一種涵蓋多面向的概念，包括貨架期間的物理、生理、營養及病理變化，因此，評鑑優良農產品的品質需檢測外觀 (大小、形狀、顏色、光澤及無缺陷)、質地 (硬度與脆度)、風味 (甜度、酸度及香氣)，及營養價值 (維生素、礦物質及營養成分)。

Ulrich & Olbricht (2016) 曾探討消費者喜好程度 (接受度) 與草莓果實 SSC、酸度及香

氣的關聯性，結果發現『接受度』與複合『芳香』及『甜味』兩關鍵因子呈正相關，而與草莓『甜味』有關的揮發性化合物有 2–甲基丁酸甲酯 (methyl 2–methylbutyrate)、芳樟醇 (linalool) 及癸酸 (decanoic acid) 等物質；有些研究也指出，果實中相對較高的 SSC (8.5–10.0°Brix) 與可滴定酸 (titratable acid; TA) 比值，與消費者味覺感知接受度的相關性，比起單獨評估 SSC 或 TA 更高，顯示消費者對兼具甜度、酸度、香氣及質地的草莓品種有更好的接受程度 (Saad *et al*. 2022; Castillo-Girones *et al*. 2025)。本試驗將溫室內栽培的「桃園 1 號」、「台農 1 號」及「香水」三個品種收穫的果實依重量分成 4 個等級，再依序調查其平均鮮重、果肩寬、果長、硬度、SSC 及酸度等與品質相關的數據 (表 3)。綜合檢測結果顯示，「台農 1 號」果實硬度約為 0.8–0.9 kg cm^{-2}，比「桃園 1 號」(0.6 –0.7 kg cm^{-2}) 與「香水」(0.6–0.7 kg cm^{-2}) 高，Huber (1984) 認為果實硬度與果皮韌性、果膠及半纖維素含量或蛋白質累積有關。因此，果實具備適當硬度將有助於顯現良好的口感。「桃園 1 號」果實的 SSC 介於 8.5–11.8°Brix，與「台農 1 號」的 9.5–10.4°Brix 數值相近，但高於「香水」果實的 6.2–7.4°Brix；另檢測各供試品種果實的酸度，則是「桃園 1 號」(0.34–0.44%) 比「台農 1 號」(0.28–0.36%) 與「香水」(0.27–0.31%) 稍高，由此估算「桃園 1 號」與「台農 1 號」的 SSC 與酸度比值較「香水」為佳。已知草莓 SSC 主要係以葡萄糖與果糖含量為主，蔗糖含量偏低，且果酸來源多屬檸檬酸與蘋花酸等成分 (Hancock *et al*. 2008)，若再參考前述幾位學者的論點，則評估消費者對於「桃園 1 號」與「台農 1 號」的接受度應該會比「香水」佳；不過，考量在本所設施試驗環境下收穫之「香水」各分級果實平均鮮重皆比「桃園 1 號」與「台農 1 號」高，雖然在統計上未有顯著差異，但實際栽培後之單果數量較多，此對於某些栽種草莓的農友而言，或許會選擇較高產量的品種以期增加果品銷售收入。

草莓果實內含的揮發性物質是香氣主要來源，包括 δ–戊內酯 (δ–valerolactone) 與草莓呋喃酮 (furanone) 等約 360 種化合物 (Pérez *et al*. 1996, 1999)，酯類化合物賦予草莓豐富的果香與花香，這些物質雖僅占鮮重的 0.001–0.01%，但對品質卻有重大影響 (Forney *et al*. 2000)。Chen & Shiau (2020) 曾報導指出「桃園 1 號」與「台農 1 號」果實香味強，「香水」則屬中等；Chang & Song (2013) 也鑑定出「桃園 1 號」與「香水」的香氣成分，其中「桃園 1 號」含乙酸乙酯 (ethyl acetate) 與β-芳樟醇 (β-linalool) 等化合物，更具濃郁的蜜糖香與果香；「香水」則因含有匙葉桉油烯醇 (spathulenol)，故略有類似於藥草的氣味。由於檢測香氣成分需使用氣相層析儀 (gas chromatography; GC) 與氣相層析質譜儀 (gas chromatography-mass spectrometry; GC-MS) 等貴重儀器與專業技術，因此，本試驗並未分析「台農 1 號」香氣的組成分，但未來將積極與學者合作進行相關研究。

由於全球持續暖化與氣候變遷，使得國內夏、秋季高溫期變長，導致一些草莓品種在育苗期與生育期發生病害比率劇增，Wu *et al*. (2022) 研究指出，草莓「桃園 1 號」為炭疽病的感病品種，「香水」與「台農 1 號」葉部對於 *Colletotrichum siamense* ML133 與 *C. fructicola* ML356 等炭疽病菌則有相對抗性；不過，Wu *et al*. (2021) 也發現「香水」容易受到由 *Neopestalotiopsis rosae* 引起的葉枯病嚴重危害；本試驗則是將採集自大湖地區的白粉病菌株 *Podosphaera aphanis* (舊名：*Sphaerotheca macularis* f. sp. *fragariae*) 接種至前述 3 個供試品種苗株，並參考 Amsalem *et al*. (2006) 報導有關適合白粉病菌株孢子萌發的環境溫度約為 15–25℃，RH 為 70–75%，因此，本試驗所使用的植物生長箱溫度與溼度均調整為適合白粉病發病的條件；另因此種病原菌屬於絕對寄生，故一般在進行菌種鑑別與抗病性試驗時，多會採取罹病葉片摩擦健康且幼嫩的葉片 (Sargent *et al*. 2019)，或利用噴霧接種方式令供試植株感病後，再評估葉面感染嚴重程度。已知「香水」葉片在整個試驗過程皆未觀察到病斑，顯示對白粉病具有良好的抗病能力；「桃園 1 號」葉片則出現不同程度的白粉病病斑，且平均罹病率達 22.3% (圖 3)，此與 Lee *et al*. (1993) 調查之 19.1% 相近，故可視為感病品種；至於，「台農 1 號」葉片整

體罹病率僅為 4.5%，顯著低於「桃園 1 號」的罹病率，且與「香水」(0%) 比較後無顯著差異，可見其對白粉病也具有一定程度的抗性，這項結果是首度經由試驗取得及發表，益顯資料之重要。

綜合上述各項結果可知，在本所設施試驗環境下培育的「台農 1 號」草莓，植株形態屬於半直立型且果實外觀大多呈深紅色圓錐形 (圖 2)，不但有適當的硬度與良好口感，並具備與「桃園 1 號」(父本) 相似的濃郁香氣，雖然產量略低於香氣中等的「香水」(母本)，但兼具白粉病與炭疽病的相對抗性。因此，仍符合當初制訂的育種目標且獲得的資料亦能提供農友們參考。我國現今正積極推行草莓健康種苗 3 級繁殖制度，各階段種苗不得檢出炭疽病 (*Colletotrichum* spp.)、萎凋病 (*Fusarium oxysporum* f. sp. *fragariae*) 及根腐線蟲 (*Pratylenchus* sp.)。本次研究僅先聚焦於白粉病抗性，未來將持續探討包含炭疽病與葉枯病等多種病害之抗性，以提供更完整的抗病性資料。目前，包含「台農 1 號」在內的 7 個市售品種，已逐步建立健康種苗繁殖體系，期望「台農 1 號」健康種苗積極推廣後，能讓農友們在栽培時多一種選擇，且栽種後能獲得理想的收益，而廣大消費群眾也可以品嘗到風味甚佳的國產優質草莓。

誌謝

本試驗歷年承農業部主管科技計畫如 112 農科-4.6.1-農-C2、113 農科-4.6.1-農-02 及 114 農科-4.6.1-農-01 等經費補助，謹此申謝。

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Studies on Horticultural Characteristics and Powdery Mildew Resistance of Strawberry ‘Tainung No. 1’ and Its Parents

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Abstract

Shiau, Y. J., T. C. Lin, and Y. J. Lee. 2026. Studies on horticultural characteristics and powdery mildew resistance of strawberry ‘Tainung No. 1’ and its parents. J. Taiwan Agric. Res. 75(1):135−146.

Fragaria ×ananassa Duch. ‘Tainung No. 1’ is a variety bred through cross-pollination between ‘Xiang-Shui’ (female parent) and ‘Taoyuan No. 1’ (male parent). This study aimed to investigate the horticultural characteristics of ‘Tainung No. 1’ and its two parents and to test their resistance to powdery mildew. The results showed that, under the test conditions, the plants of ‘Tainung No. 1’ (18.5 cm) and ‘Xiang-Shui’ (18.6 cm) were significantly taller than those of ‘Taoyuan No. 1’ (14.8 cm). Furthermore, the plant width of ‘Taoyuan No. 1’ (39.3 cm) was greater than that of ‘Tainung No. 1’ (37.5 cm) and ‘Xiang-Shui’ (36.8 cm). The number of stolon (7.6), terminal leaflet leaf length (10.6 cm), leaf width (10.2 cm), and trifoliolate leaf area (308.6 cm^2) of ‘Taoyuan No. 1’ per plant were significantly larger than those of the other two varieties, but the petiole length (11.0 cm) was the shortest. The average total number of inflorescence per plant (7.1), the number of fruits per inflorescence (12.7), and the average fresh weight of fruits (13.2 g) for ‘Xiang-Shui’ were all higher than those of ‘Tainung No. 1’ and ‘Taoyuan No. 1’. However, the fruit hardness of ‘Tainung No. 1’ was higher (0.8–0.9 kg cm^{-2}), and the soluble solids content (9.5–10.4°Brix) was similar to that of ‘Taoyuan No. 1’ (8.5–11.8°Brix), while that of ‘Xiang-Shui’ (6.2–7.4°Brix) was lower. The ‘Taoyuan No. 1’ has a higher fruit acidity of 0.34–0.44% than the other two varieties, but its resistance to powdery mildew is not as good as ‘Xiang-Shui’ and ‘Tainung No. 1’. These results may provide farmers with a reference for cultivating strawberries.

Key words: Disease resistance, *Fragaria ×ananassa* Duch. ‘Tainung No. 1’, *Fragaria ×ananassa* Duch. ‘Taoyuan No. 1’, *Fragaria ×ananassa* Duch. ‘Xiang-Shui’, Powdery mildew.

Receiverd: September 12, 2025; Accepted: November 25, 2025.

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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):147–157 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0009

Research Article

Comparative Evaluation of Antioxidant and Anti-Inflammatory Activities of Water Extracts from Major Taiwanese *Mesona chinensis* Cultivars

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Abstract

Hung, T. H., C. H. Chen, C. H. Chang, Y. L. Lee, Y. M. Yeh, T. L. Kung, and C. Huang. 2026. Comparative evaluation of antioxidant and anti-inflammatory activities of water extracts from major Taiwanese *Mesona chinensis* cultivars. J. Taiwan Agric. Res. 75(1):147–157.

Mesona chinensis Benth. water extracts (MWE) have traditionally been used in Taiwan for health-promoting purposes, yet the comparative bioactivities of major cultivars remain unclear. This study examined four representative cultivars: ‘Taoyuan No. 1’ (‘TY1’), ‘Taoyuan No. 2’ (‘TY2’), ‘TARI No. 1’ (‘TN1’), and ‘Shuishang’ (‘SS’), to compare their total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, and anti-inflammatory effects. Among them, ‘TY2’ exhibited the highest TPC, TFC, and antioxidant capacity, followed by ‘TY1’, whereas ‘TN1’ and ‘SS’ showed lower levels. All water extracts were non-cytotoxic to RAW264.7 macrophages at concentrations up to 500 μg mL^{-1} and slightly enhanced cell viability. All cultivars significantly suppressed lipopolysaccharide (LPS)-induced nitric oxide (NO) production, and the degree of inhibition roughly corresponded with their antioxidant capacity. In contrast, TNF-α and IL-6 levels were not significantly affected, and mild elevations were observed in some cultivars. Collectively, these findings demonstrate that MWE possess NO-suppressing anti-inflammatory potential. This study provides a comparative framework for identifying cultivars with superior functional characteristics and future value in functional food development.

Key words: *Mesona chinensis* water extract, Antioxidant, Nitric oxide, TNF-α, IL-6.

Received: September 26, 2025; Accepted: November 14, 2025.
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INTRODUCTION

Mesona chinensis Benth. (commonly known as grass jelly) is a traditional medicinal and edible herb of the Lamiaceae family, widely consumed in Taiwan and other Asian regions (Seah *et al.* 2024). Its characteristic cooling flavor has made it a popular summertime refreshment, but its value extends beyond its culinary appeal. Phytochemical analyses have revealed that *M. chinensis* is rich in bioactive compounds- particularly phenolic acids and flavonoids- associated with antioxidant, anti-inflammatory, hypoglycemic, and uric acid-lowering activities (Hung & Yen 2002; Lo *et al.* 2003; Chusak *et al.* 2014; Jhang *et al.* 2016). These findings provide scientific support for the plant's long-standing traditional uses and open opportunities for applications in functional foods and preventive health care.

Inflammation is a fundamental defense mechanism against infection or tissue injury (Megha *et al.* 2021). However, chronic or uncontrolled inflammation is a recognized risk factor for cardiovascular disease, diabetes, cancer, and neurodegenerative disorders (Coussens & Werb 2002; Wellen & Hotamisligil 2005; DeLegge & Smoke 2008; Pawelec *et al.* 2014). As a result, there is growing interest in natural products with anti-inflammatory potential as safer alternatives or complements to conventional therapeutics (Serrano *et al.* 2018; Merecz-Sadowska *et al.* 2020; Xie *et al.* 2024).

Several inflammatory mediators play central roles in this process. Nitric oxide (NO) contributes both to vascular regulation and to inflammatory signaling. Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are key pro-inflammatory cytokines whose overproduction drives tissue damage and chronic inflammatory states (Zelová & Hošek 2013; Tanaka *et al.* 2014; Man *et al.* 2022; Russo *et al.* 2023). Extracts of *M. chinensis* have been reported to suppress these mediators in various experimental models (Huang *et al.* 2012; Huang *et al.* 2021; Huang *et al.* 2022; Seah *et al.* 2024).

Taiwan has a long history of cultivating and consuming *M. chinensis* (Hsieh *et al.* 2020), and four cultivars dominate domestic production: 'Taoyuan No. 1' ('TY1'), 'Taoyuan No. 2' ('TY2'), 'TARI No. 1' ('TN1'), and 'Shuishang' ('SS') (Hu *et al.* 2000; Hung *et al.* 2025). Despite the recognized health-promoting properties of *M. chinensis*, no systematic comparison of the antioxidant and anti-inflammatory activities of these major Taiwanese cultivars has been reported.

Water extracts were selected as the study material because previous research has shown that *M. chinensis* aqueous extracts exhibit stronger anti-inflammatory activity than ethanol extracts (Fan *et al.* 2021). Building on this rationale, we evaluated the total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, and inhibition of inflammatory mediators (NO, TNF-α, and IL-6) of water extracts prepared from the four cultivars. By linking phytochemical profiles with bioactivity outcomes, this work provides a scientific basis for identifying cultivars with superior functional potential and supports future breeding, cultivation, and product development strategies targeting the anti-inflammatory properties of *M. chinensis*.

MATERIALS AND METHODS

Plant materials

M. chinensis cultivars 'TY1', 'TY2', 'TN1', and 'SS' were all purchased from farmers in Shuishang Township, Chiayi County, where they were cultivated in the same field (GPS: 23.418274°N, 120.430018°E). Stems and leaves were harvested together and sun-dried until the moisture content was confirmed to be below 10%. The dried materials were then stored at room temperature for six months prior to analysis. All voucher materials (Batch No.: TARI-23M1-M4) have been deposited at the Taiwan Agricultural Research Institute for future reference.

Extraction procedure

The dried *M. chinensis* material was ground using a tabletop mill (Chyun Tseh, Taichung, Taiwan) and passed through a 20-mesh sieve. The resulting powder was mixed with distilled

water at a ratio of 1:20 (w/v) and extracted using an ultrasonic extractor (ES-600N, TST, Taiwan; frequency 40 kHz, power 600 W) at 80℃ for 1 h. After extraction, the mixture was centrifuged at 14,000 g, and the supernatant was collected and lyophilized using a freeze dryer (FD-25B3P8, HCS, Taipei, Taiwan) to obtain the *M. chinensis* water extract (MWE), which was stored at -20℃ until analysis.

Total phenolic content (TPC)

TPC was determined following the Folin–Ciocalteu method (Prior *et al.* 2005). In brief, MWEs were mixed with diluted Folin-Ciocalteu reagent (109001, Merck, Darmstadt, Germany), followed by addition of sodium carbonate solution (31432, Honeywell, Offenbach am Main, Germany) for color development. Absorbance was measured at 750 nm using a microplate reader. Results were expressed as mg gallic acid equivalents per g extract (mg GAE g^{-1}) based on a gallic acid (410862500, Acros Organics, Geel, Belgium) calibration curve.

Total flavonoid content (TFC)

TFC was measured according to the method of Jing *et al.* (2015) with minor modifications. MWEs were sequentially reacted with sodium nitrite (106549, Merck, Darmstadt, Germany), aluminum chloride (0528-01, J.T.Baker, Phillipsburg, NJ, USA), and sodium hydroxide solutions. Absorbance was read at 510 nm using a microplate reader. Results were expressed as mg rutin equivalents per g extract (mg RUE g^{-1}) using a rutin (19868, Cayman, Ann Arbor, MI, USA) standard curve.

ABTS radical scavenging assay

Antioxidant activity was evaluated using the 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) scavenging assay as described by Re *et al.* (1999). Briefly, an $ABTS^{\cdot+}$ (A3219, Sigma-Aldrich, St. Louis, MO, USA) radical solution was prepared and diluted to the desired absorbance, then mixed with the MWE or water (control). Absorbance was immediately recorded at 734 nm using a microplate reader. Antioxidant capacity was quantified against a Trolox (238813, Sigma-Aldrich, St. Louis, MO, USA) standard curve and expressed as mg Trolox equivalents per g extract (mg TE g^{-1}).

Cell viability assessment (CCK-8 Assay)

Cell viability was evaluated using the Cell Counting Kit-8 (CCK-8) assay (CK04, Dojindo, Kumamoto, Japan) following the method of Tang *et al.* (2019) with minor modifications. Mouse macrophage RAW 264.7 cells (Bioresource Collection and Research Center, Hsinchu, Taiwan) were seeded into 96-well plates at a density of 1.8×10^4 cells per well. Cells were treated for 24 h with MWE. The control wells were received an equivalent volume of extraction solvent. At the end of treatment, 10 µL of CCK-8 solution was added to each well and the plates were incubated at 37℃ for 20 min. Absorbance was then measured at 450 nm using an ELISA reader. Cell viability was expressed as a percentage relative to the untreated control.

LPS-Induced inflammatory response

The procedure for inducing an inflammatory response was adapted from Tang *et al.* (2019). RAW 264.7 cells (8×10^4 cells per well) were seeded into 48-well culture plates and maintained in Dulbecco's modified Eagle medium (DMEM) (Gibco, USA) at 37℃ in a humidified incubator with 5 % CO_2. After 24 h of culture, lipopolysaccharide (LPS; 1 µg mL^{-1}; L5418, Sigma, USA) and the designated treatments were added simultaneously as follows: the experimental groups received 100 µg mL^{-1} of MWE, the positive control group received 100 µM hydrocortisone (HYD; H0888, Sigma-Aldrich, St. Louis, MO, USA), and the inflammation control group received no additional treatment. Cells without LPS stimulation served as the negative control. Culture supernatants were collected at two time points: 100 µL at 6 h post-LPS stimulation for TNF-α measurement, and another 100 µL at 24 h post-LPS stimulation for NO and IL-6 assays. All collected samples were stored at -80℃ until analysis.

Measurement of inflammatory mediators

The determination of inflammatory mediators (NO, TNF-α, and IL-6) was performed following a protocol modified from Tang *et al.* (2019). NO levels were quantified using the Griess reagent method. Briefly, 0.4 g of Griess reagent (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 10 mL of deionized water to prepare the working solution. The second aliquot of supernatant was mixed with an equal volume of Griess reagent and incubated at room temperature for 15 min, and the absorbance was read at 540 nm using an ELISA plate reader.

TNF-α levels were determined using the ELISA MAX™ Deluxe Set Mouse TNF-α kit (BioLegend, San Diego, CA, USA). The first aliquot of supernatant was diluted 1:100 and added to ELISA plates pre-coated with capture antibody, followed by incubation at room temperature for 2 h. After washing, detection antibody was added and incubated for 1 h at room temperature. The plates were washed again, substrate solution was applied, and absorbance was measured at 450 nm.

IL-6 levels were measured using the ELISA MAX™ Deluxe Set Mouse IL-6 kit (BioLegend, San Diego, CA, USA). The second aliquot of supernatant was diluted 1:100 and added to ELISA plates pre-coated with IL-6 capture antibody, followed by 2 h incubation at room temperature. After washing, detection antibody was added and incubated for 1 h. The plates were washed again, substrate solution was applied, and absorbance was measured at 450 nm using an ELISA plate reader.

Statistical analysis

All statistical analyses were performed using R (version 4.5.0; R Core Team, Vienna, Austria). Comparisons among groups were conducted using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test for post hoc pairwise comparisons. Results are expressed as mean ± standard error of the mean (SEM), and a $P < 0.05$ was considered statistically significant.

RESULTS

Total phenolic and flavonoid contents of MWE

The TPC of MWE differed significantly among the four cultivars ($P < 0.001$) (Fig. 1). 'TY2' showed the highest level (132.71 ± 1.35 mg GAE g^{-1}), followed by 'TY1' (123.67 ± 2.11 mg GAE g^{-1}), 'SS' (106.76 ± 1.78 mg GAE g^{-1}), and 'TN1' (96.05 ± 2.22 mg GAE g^{-1}).

The TFC also differed significantly among the cultivars ($P < 0.001$) (Fig. 2). 'TY1' (155.14 ± 0.65 mg RUE g^{-1}) and 'TY2' (153.31 ± 1.06 mg RUE g^{-1}) were significantly higher than 'SS' (125.40 ± 0.52 mg RUE g^{-1}) and 'TN1' (125.19 ± 1.65 mg RUE g^{-1}). No significant difference was detected between 'TY1' and 'TY2', or between 'SS' and 'TN1'.

Antioxidant capacity of MWE

The antioxidant capacity of the MWE varied significantly among cultivars ($P < 0.001$) (Fig. 3). 'TY2' exhibited the highest activity (199.93 ± 1.63 mg TE g^{-1}), followed by 'TY1' (191.40 ± 1.72 mg TE g^{-1}). 'TN1' (155.70 ± 1.51 mg TE g^{-1}) and 'SS' (151.83 ± 1.86 mg TE g^{-1}) showed markedly lower values.

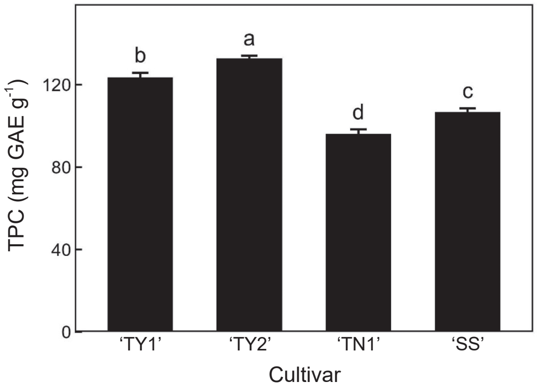

Fig. 1. Total phenolic content (TPC) of MWE from 4 cultivars. TPC was measured by the Folin-Ciocalteu method and expressed as gallic acid equivalents (mg GAE g^{-1}). 'TY1': 'Taoyuan No. 1'; 'TY2': 'Taoyuan No. 2'; 'TN1': 'TARI No. 1'; 'SS': 'Shuishang'. Data represent mean ± *SE* ($n = 3$). Different letters above bars indicate significant differences among cultivars ($P < 0.05$, Tukey's test).

Effects of MWE on RAW264.7 cell viability

Across the tested concentration range (0–500 μg mL^{-1}), MWE from all four cultivars increased RAW264.7 cell viability relative to the untreated control (Fig. 4). 'TY1' and 'TY2' displayed a clear concentration-dependent rise, with mean viability exceeding 1.2-fold of control at 500 μg mL^{-1}. 'TN1' and 'SS' showed small fluctuations at some intermediate doses, but their overall pattern likewise trended upward as extract concentration increased. No decrease below the control level was detected at any dose.

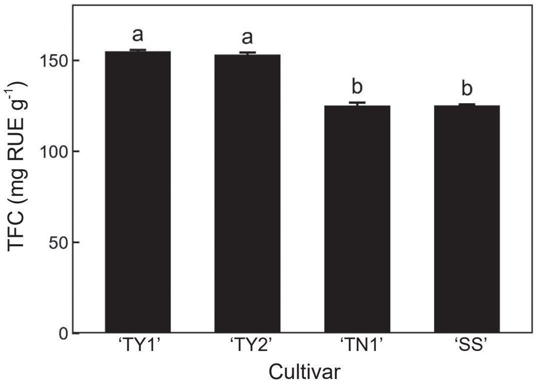

Fig. 2. Total flavonoid content (TFC) of MWE from 4 cultivars. TFC was determined by a colorimetric method based on Jing *et al*. (2015) and expressed as rutin equivalents (mg RUE g^{-1}). 'TY1': 'Taoyuan No. 1'; 'TY2': 'Taoyuan No. 2'; 'TN1': 'TARI No. 1'; 'SS': 'Shuishang'. Data represent mean ± *SE* (n = 3). Different letters above bars indicate significant differences among cultivars ($P < 0.05$, Tukey's test).

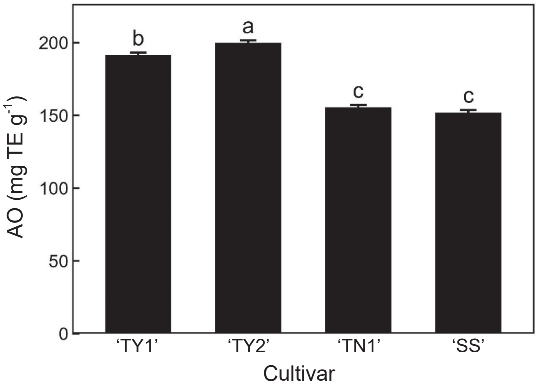

Fig. 3. Antioxidant (AO) capacity of *Mesona chinensis* water extract (MWE) from four cultivars determined by ABTS radical scavenging assay and expressed as Trolox equivalents (mg TE g^{-1}). 'TY1': 'Taoyuan No. 1'; 'TY2': 'Taoyuan No. 2'; 'TN1': 'TARI No. 1'; 'SS': 'Shuishang'. Data represent mean ± *SE* (n = 3). Different letters above bars indicate significant differences among cultivars ($P < 0.05$, Tukey's test).

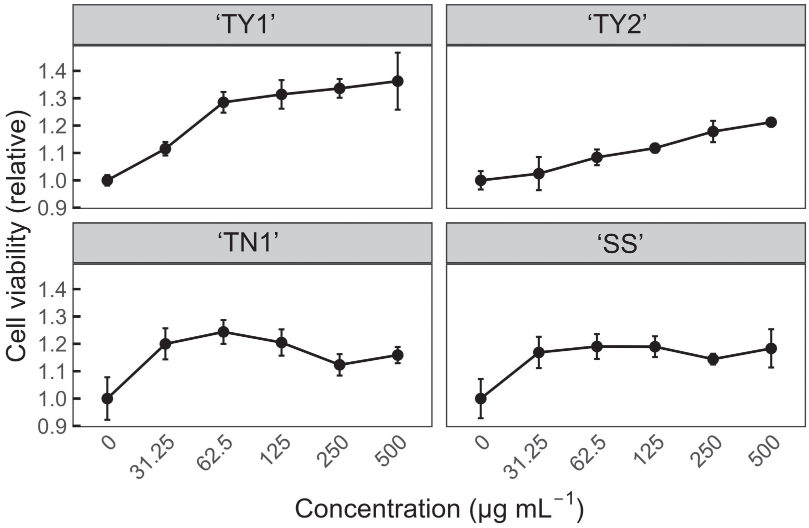

Fig. 4. Effects of *Mesona chinensis* water extract (MWE) from four cultivars ('TY1': 'Taoyuan No. 1'; 'TY2': 'Taoyuan No. 2'; 'TN1': 'TARI No. 1'; 'SS': 'Shuishang') on RAW264.7 cell viability after 24 h exposure, determined by the CCK-8 assay. Cells were treated with different concentrations of extracts (0–500 μg mL^{-1}). Data are expressed as mean ± *SD* (n = 4).

Effects of MWE on LPS-induced inflammatory mediators

LPS significantly increased the production in all inflammatory mediators (NO, TNF-α, and IL-6) compared with the untreated control (C group), while the positive-control HYD group effectively suppressed these inflammatory mediators ($P < 0.05$; Figs. 5–7), confirming the responsiveness of the RAW264.7 macrophage model.

MWE from the four cultivars exhibited differential inhibitory effects on LPS-induced NO production (Fig. 5). 'TY2' showed the strongest inhibition (64.5%), followed by 'TN1' (36.7%) and 'SS' (35.7%), whereas 'TY1' exerted only a mild effect (11.7%). These results indicate that NO suppression varied among cultivars, with 'TY2' being the most effective. Detailed group comparisons are provided in Fig. 5.

In contrast, MWE did not inhibit TNF-α levels compared with the LPS group (Fig. 6). 'TY2' showed a small reduction (11.8%), but this was not statistically significant. 'TY1' and 'SS' were similar to LPS. 'TN1' even significantly increased TNF-α production. These results indicate that MWE treatment did not exert an inhibitory effect on TNF-α in this model.

IL-6 production was not inhibited by MWE either (Fig. 7). 'TY1', 'TY2', and 'TN1' showed no significant change relative to LPS. In the contrast, 'SS' significantly enhanced IL-6 levels. Overall, MWE had no inhibitory effect on IL-6 production, with 'SS' even increasing its levels.

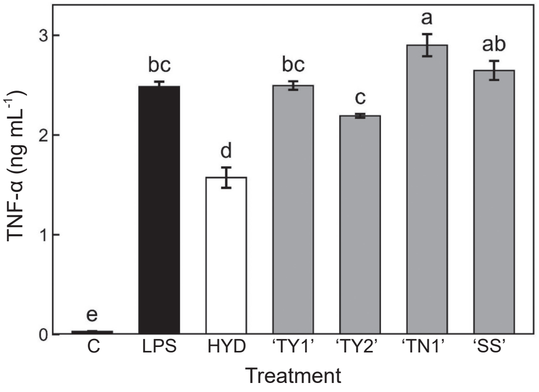

Fig. 6. Effects of *Mesona chinensis* water extract (MWE) from 4 cultivars on LPS-induced TNF-α production in RAW264.7 cells. C: control group, LPS: LPS-treated group, HYD: hydrocortisone-treated positive control; 'TY1': 'Taoyuan No. 1'; 'TY2': 'Taoyuan No. 2'; 'TN1': 'TARI No. 1'; 'SS': 'Shuishang'. Data represent mean ± *SE* ($n = 3$). Different letters above bars indicate significant differences among groups ($P < 0.05$, Tukey's test).

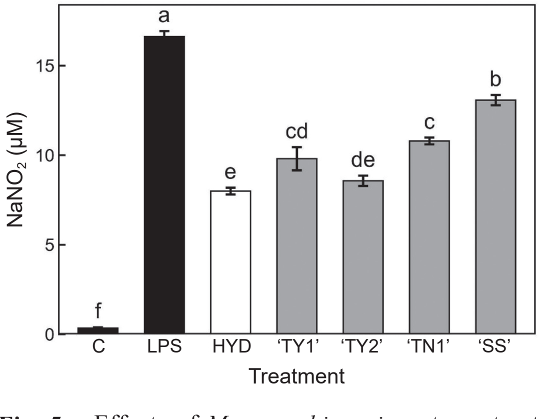

Fig. 5. Effects of *Mesona chinensis* water extract (MWE) from 4 cultivars on LPS-induced NO production in RAW264.7 cells. C: control group; LPS: LPS-treated group; HYD: hydrocortisone-treated positive control; 'TY1': 'Taoyuan No. 1'; 'TY2': 'Taoyuan No. 2'; 'TN1': 'TARI No. 1'; 'SS': 'Shuishang'. Data represent mean ± *SE* ($n = 3$). Different letters above bars indicate significant differences among groups ($P < 0.05$, Tukey's test).

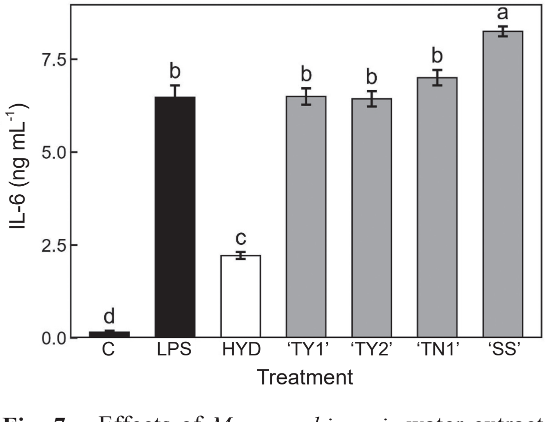

Fig. 7. Effects of *Mesona chinensis* water extract (MWE) from 4 cultivars on LPS-induced IL-6 production in RAW264.7 cells. C: control group, LPS: LPS-treated group, HYD: hydrocortisone-treated positive control; 'TY1': 'Taoyuan No. 1'; 'TY2': 'Taoyuan No. 2'; 'TN1': 'TARI No. 1'; 'SS': 'Shuishang'. Data represent mean ± *SE* ($n = 3$). Different letters above bars indicate significant differences among groups ($P < 0.05$, Tukey's test).

DISCUSSION

Antioxidant profile of MWE

Previous studies have shown that MWE possess strong antioxidant capacity, largely attributed to phenolic acids and flavonoids (Hung & Yen 2002). However, no study has systematically compared the TPC, TFC, and antioxidant capacity among the four major Taiwanese cultivars. The present work addresses this gap.

Our results reveal marked cultivar-dependent differences in TPC, TFC, and antioxidant capacity. In general, antioxidant capacity was positively associated with both TPC and TFC. 'TY2' showed the highest antioxidant activity, followed by 'TY1', whereas 'TN1'and 'SS' exhibited lower levels of both phenolics and antioxidant potential. Nevertheless, this correlation was not strictly linear, implying that qualitative variation in phenolic composition may also contribute to antioxidant performance.

Interestingly, a study using 50% ethanol extraction reported that 'SS' and 'TN1'contained the highest levels of rosmarinic acid, a strong antioxidant, among the four cultivars (Hung *et al*. 2025), whereas the present water extract results showed relatively low antioxidant activity in these same cultivars. This discrepancy highlights the solvent-dependent recovery of bioactive constituents. Water extraction is generally less efficient than hydroalcoholic solvents for moderately polar compounds such as rosmarinic acid and flavonoid glycosides (Ziani *et al*. 2023). Consequently, the antioxidant activity of water extracts may instead arise mainly from more hydrophilic phenolic acids, such as caffeic, protocatechuic, vanillic, and syringic acids (Hung & Yen, 2002; Xiao *et al*. 2022), along with polysaccharides that have been reported to possess radical-scavenging activity (Lin *et al*. 2017).

Further studies should quantify these hydrophilic components to clarify their relative contributions to the overall antioxidant potential of *M. chinensis* cultivars. Although the present work focused on total phenolic and flavonoid contents, further chromatographic profiling using high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS) of individual compounds will be necessary to better elucidate the chemical determinants of cultivar-specific antioxidant and anti-inflammatory activities.

Cytotoxicity assessment and NO suppression of MWE

CCK-8 assays confirmed that MWE from all four cultivars were not cytotoxic to RAW264.7 macrophages at concentrations up to 500 µg mL^{-1}. A slight increase in cell viability was even observed across all extracts, suggesting that these water extracts are biologically safe for immune cells (Fig. 4). The LPS challenge successfully induced NO, TNF-α, and IL-6 production, while HYD effectively suppressed these responses, validating the reliability of the RAW264.7 model and confirming that our positive control functioned as expected (Figs. 5–7).

Suppression of NO is a recognized indicator of anti-inflammatory potential. All *M. chinensis* cultivars significantly reduced LPS-induced NO production (Fig. 5). The strength of this inhibition seemed to track with the extracts' antioxidant capacity. For example, 'TY2' had the highest antioxidant activity, and also produced the strongest NO suppression. In the contrast, 'TN1' and 'SS' with weaker antioxidant activity showed correspondingly weaker NO inhibition. These findings are consistent with the view that the antioxidant capacity of natural extracts is associated with the ability of NO suppression (Abas *et al*. 2006; Bor *et al*. 2006).

Mechanistically, the observed NO suppression by MWE may result from downregulation of inducible nitric oxide synthase (iNOS) or modulation of upstream signaling pathways such as MAPK–NF-κB. Supporting evidence comes from prior studies in which aqueous *M. chinensis* extracts lowered iNOS expression in carrageenan-induced models (Huang *et al*. 2012). In addition, triterpenes and sesquiterpenes isolated from *M. chinensis* methanolic extracts potently inhibited

LPS-induced NO production in RAW264.7 cells, concomitantly reducing iNOS protein levels through MAPK–NF-κB pathway interference (Huang *et al*. 2021; Huang *et al*. 2022). Future work should examine whether cultivar-to-cultivar differences in NO suppression are associated with variations in iNOS/MAPK–NF-κB signaling. Moreover, because our data showed a positive correlation between antioxidant capacity and NO suppression, identifying the specific antioxidant compounds responsible will be crucial for assessing anti-inflammatory potential of *M. chinensis*.

Complexity in TNF-α and IL-6 modulation of MWE

TNF-α and IL-6 are key pro-inflammatory cytokines governed by NF-κB, and their sustained production amplifies inflammatory responses. Under our water-extraction conditions, MWE did not significantly suppress TNF-α or IL-6 production in RAW264.7 cells (Figs. 6–7). Instead, specific cultivars triggered pro-inflammatory cytokines: 'TN1' increased TNF-α and 'SS' elevated IL-6. Similar patterns have been reported for some natural extracts such as ethanol extract of *Andrographis paniculata* (Burm. f.) Wall. ex Nees and hexane extract of *Taxillus chinensis* Chiu, with strong inhibition of NO but little or no effect on TNF-α or IL-6 (Chao *et al*. 2007).

Many reports of reduced TNF-α and IL-6 levels after *M. chinensis* treatment involve organic-solvent extracts or isolated terpenes (Huang *et al*. 2021; Huang *et al*. 2022). In addition, phenolic acids abundant in *M. chinensis* such as rosmarinic acid and caffeic acid have been demonstrated to directly down-regulate pro-inflammatory mediators (Qiao *et al*. 2005; Migliori *et al*. 2015). By contrast, polysaccharide-rich water extracts have frequently (though not always) been reported to increase TNF-α and IL-6 secretion in macrophages. For example, *M. chinensis* polysaccharides were shown to raise IL-6, IL-1β, and TNF-α in macrophages (Huang *et al*. 2020), and sulfated *M. chinensis* polysaccharide enhanced RAW264.7 viability together with IL-6, IL-1β, and TNF-α secretion (Shen *et al*. 2021). Collectively, these findings indicate that crude water extracts may contain chemical classes with opposing biological effects. The immunostimulatory fraction likely includes crude polysaccharides, which account for about 7% of the dry sample (Lin *et al*. 2017). In contrast, phenolic acids and triterpenes are recognized for their anti-inflammatory activity. Therefore, the net anti-inflammatory activity of each cultivar will reflect the balance between these chemical groups and depend on both cultivar genetics and extraction parameters.

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臺灣主要仙草品種水萃物之抗氧化與抗發炎活性比較

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摘要

洪子桓、陳秋樺、張家華、李雅琳、葉永銘、龔財立、黃琤。2026。臺灣主要仙草品種水萃物之抗氧化與抗發炎活性比較。台灣農業研究 75(1):147–157。

仙草 (*Mesona chinensis* Benth.) 水萃物傳統上具有保健功效，但針對臺灣主要品種的抗氧化與抗發炎活性尚缺乏系統性比較。本研究評估 4 個仙草品種：「桃園 1 號」(‘TY1’)、「桃園 2 號」(‘TY2’)、「農試 1 號」(‘TN1’) 及「水上種」(‘SS’) 的總酚含量 (total phenolic content; TPC)、總黃酮含量 (total flavonoid content; TFC)、抗氧化能力以及抗發炎活性。結果顯示，‘TY2’ 的 TPC、TFC 及抗氧化力最高，其次為 ‘TY1’，而 ‘TN1’ 與 ‘SS’ 較低。所有水萃物在 RAW264.7 巨噬細胞中於 500 μg mL^{-1} 以下均無細胞毒性，甚至略微提升細胞活性。所有品種均顯著抑制脂多醣誘導的一氧化氮生成，且抑制強度大致與抗氧化力相關。相比之下，腫瘤壞死因子 α 與介白素 -6 生成並未被仙草水萃物明顯抑制，部分品種 (‘TN1’ 與 ‘SS’) 甚至促進其分泌。整體而言，仙草水萃物具有抑制一氧化氮的抗發炎活性。本研究為篩選具最佳抗氧化及抗發炎潛力的仙草品種提供了比較框架。

關鍵詞：仙草水萃物、抗氧化、一氧化氮、腫瘤壞死因子 α、介白素 -6。

投稿日期：2025 年 9 月 26 日；接受日期：2025 年 11 月 14 日。
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台灣農業研究 (J. Taiwan Agric. Res.) 75(1):159–169 (2026)
https://doi.org/10.6156/JTAR.202603_75(1).0010

Research Article

Inhibition Potential and Molecular Mechanisms of Longan Flower Water Extract in Lung Adenocarcinoma Cells

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Abstract

Hung, T. H., W. L. Qiu, Z. H. Lin , A. J. Tseng, and T. Y. Lin. 2026. Inhibition potential and molecular mechanisms of longan flower water extract in lung adenocarcinoma cells. J. Taiwan Agric. Res. 75(1):159–169.

Lung adenocarcinoma is the most common subtype of non-small cell lung cancer, characterized by high incidence and poor survival. Natural plant-derived compounds offer potential sources for anticancer drug development. This study investigated the inhibition effects and molecular mechanisms of longan flower water extract (LFWE) in H1975 lung adenocarcinoma cells. LFWE significantly reduced H1975 cell viability and induced notable morphological changes. Mechanistically, LFWE activated autophagy, evidenced by increased LC3-II/LC3-I ratios and decreased P62 levels, and promoted lysosome-mediated degradation of mutant epidermal growth factor receptor (EGFR), leading to inhibition of downstream mTOR signaling. However, LFWE showed a degree of inhibition activity toward non-malignant lung cells, potentially imposing limitations. Further studies on its safety and translational application are necessary. In summary, this study provides preliminary evidence for exploring LFWE as a potential therapeutic strategy for lung adenocarcinoma.

Key words: Longan flower water extract, Lung adenocarcinoma, Autophagy, EGFR.

INTRODUCTION

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of cases (Leiter *et al*. 2023). Despite advances in targeted therapy and immunotherapy, the overall five-year survival rate remains low (Luo *et al*. 2022). Among NSCLC subtypes, lung adenocarcinoma is the most prevalent, frequently driven by oncogenic alterations such as mutations in epidermal growth factor receptor (EGFR), Kirsten rat sarcoma virus (KRAS), or anaplastic lymphoma kinase (ALK) fusions (Devarakonda *et al*. 2015; Chapman *et al*. 2016). These genetic complexities underscore the ongoing need for alternative or complementary therapeutic approaches.

Natural products have long been recognized as valuable sources of bioactive compounds with anticancer potential (Markman & Mekhail 2002; Acquaviva *et al*. 2022). Longan (*Dimocarpus longan* Lour.), a widely cultivated fruit tree in subtropical Asia, is not only an important agricultural crop but also a traditional medicinal plant. Longan flowers are abundant byproducts of cultivation, which are rich in polyphenols, flavonoids, and tannins, with demonstrated

Received: September 25, 2025; Accepted: November 5, 2025.
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antioxidant and anti-inflammatory properties (Ho *et al*. 2007; Hsieh *et al*. 2008). Bioactive constituents such as gallic acid, epicatechin, procyanidin A2, and corilagin have been identified (Hsieh *et al*. 2008; Sheu *et al*. 2016), several of which exhibit anticancer activity (Shay *et al*. 2015; Gupta *et al*. 2019). Previous studies reported inhibitory effects of longan flower extracts on colorectal, liver, cervical, and breast cancer cells (Lin *et al*. 2012).

However, the effects of longan flower extracts on lung adenocarcinoma, particularly at the mechanistic level, remain poorly understood. In this study, we systematically investigated the cytotoxicity and underlying molecular mechanisms of longan flower water extract (LFWE) in EGFR-mutant lung adenocarcinoma cells. Our findings aim to clarify whether LFWE may hold potential as adjunctive strategies for lung cancer treatment.

MATERIALS AND METHODS

Preparation of LFWE

Fresh longan flowers were purchased from local farmers (23.2755°N, 120.4908°E) in Dongshan District, Tainan, Taiwan. A voucher specimen (Batch No. TARI-L01) has been deposited at Taiwan Agricultural Research Institute for future reference. The flowers were sun-dried for approximately 3 d until moisture content was below 10%, as determined using a moisture analyzer (MX-50, A&D Company, Limited, Tokyo, Japan). The dried flowers were then extracted with 20 volumes of double-distilled water at 60℃ using ultrasonic-assisted extraction (ES-600N, Taiwan Supercritical Technology Co., Ltd, Changhua, Taiwan; frequency 40 kHz, power 600 W) for 1 h. The extract was filtered through Whatman™ No. 1 filter paper (Cytiva, Marlborough, MA, USA) to remove residues, concentrated under reduced pressure, and subsequently lyophilized into powder. The extraction yield was calculated, and the lyophilized powder was stored at -20℃ until use.

Cell culture

LFWE cytotoxicity was assessed in one lung cancer cell line and two non-malignant lung cell lines. The lung cancer cell line H1975, harboring the EGFR-L858R/T790M mutation, was purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, China). Normal lung epithelial cells (BEAS-2B) were purchased from the JCRB Cell Bank (Osaka, Japan), and normal lung fibroblasts (WI-38) were purchased from the Bioresource Collection and Research Center, Food Industry Research and Development Institute (Hsinchu, Taiwan). H1975 cells were cultured in Roswell Park Memorial Institute 1640 medium (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), while BEAS-2B and WI-38 cells were maintained in Minimum Essential Medium (Gibco, Thermo Fisher Scientific Inc., Waltham, MA, USA) containing 10% FBS. All cell lines were cultured at 37℃ in a humidified incubator with 5% CO_2 and passaged every 2–3 d.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The cytotoxic effects of LFWE on lung cancer and non-malignant lung cell lines were evaluated using the MTT assay. Cells were seeded in 96-well plates at a density of 5×10^4 cells per 100 μL per well and allowed to adhere for 24 h. They were then treated with various concentrations of LFWE (0–800 μg mL^{-1}) for 24–72 h. Cell viability was determined by adding MTT (Sigma-Aldrich Corporation, St. Louis, MO, USA) to each well and incubating at 37℃ for 4 h. The resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich Corporation, St. Louis, MO, USA), and absorbance was measured at 570 nm.

Cell cycle analysis

To determine whether LFWE regulates the cell cycle of lung cancer cells, H1975 cells were seeded in 6 cm culture dishes at a density

of 5 × 10^4 cells $dish^{-1}$ and allowed to adhere for 24 h. Cells were then treated with various concentrations of LFWE (0–400 μg mL^{-1}) for 48 h. After treatment, cells were harvested by trypsinization, collected by centrifugation at 1,500× *g* for 10 min, and washed with phosphate-buffered saline (PBS). The cell pellets were fixed in 70% ethanol at -20℃ for 30 min, followed by centrifugation to remove ethanol. Fixed cells were resuspended in 50 μL PBS containing propidium iodide (100 μg mL^{-1}; Elabscience, Wuhan, China) and 10 μL RNase A (Thermo Fisher Scientific Inc., Waltham, MA, USA), and incubated at 37℃ for 30 min in the dark. Cell cycle distribution was analyzed using a flow cytometer (CytoFLEX, Beckman Coulter Inc., Brea, CA, USA) with 10,000 events collected per sample. Data were processed and analyzed with CytExpert software (Beckman Coulter, Brea, CA, USA).

Apoptosis analysis

To evaluate whether LFWE induces apoptosis, lung cancer cell line H1975 was seeded at a density of 2.5 × 10^5 cells per dish and treated with LFWE at different concentrations (100, 200, and 400 μg mL^{-1}) for 48 h. After treatment, cells were harvested by trypsinization, washed with 1.0 mL cold PBS, and centrifuged at 1,800 rpm for 15 min. The cell pellets were then resuspended and stained using the fluorescein isothiocyanate (FITC) Annexin V/PI (propidium iodide) apoptosis detection kit (Elabscience, Wuhan, China) according to the manufacturer's protocol, followed by incubation at 37℃ for 15 min in the dark. Apoptotic cell populations were analyzed using Beckman CytoFLEX flow cytometry with 10,000 events acquired per sample. Data were processed with CytExpert software.

Western blot analysis

H1975 lung cancer cells were seeded in 6-cm culture dishes at a density of 5 × 10^5 cells and allowed to adhere for 24 h. Cells were then treated with different concentrations of LFWE (100, 200, and 400 μg mL^{-1}) for the indicated time periods. Following treatment, cells were washed with PBS containing 1% Na_3VO_4 and lysed with 40 μL of lysis buffer. Lysates were vortexed to disrupt membranes and centrifuged at 13,000× *g* for 10 min at 4℃. The supernatant (cell lysate) was collected, and protein concentrations were determined by the Bradford assay using bovine serum albumin (BSA) as a standard.

Equal amounts of protein (30 μg) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 10%) and transferred onto polyvinylidene difluoride (PVDF) membranes (PerkinElmer, Waltham, MA, USA). Membranes were blocked with 5% non-fat milk for 1 h at room temperature, then incubated overnight at 4℃ with the following primary antibodies: anti-EGFR (GTX121919, GeneTex, Inc., Irvine, CA, USA), anti-phospho-mTOR (Ser2448; GTX132803, GeneTex, Inc., Irvine, CA, USA), anti-phospho-AKT (Ser473; #4060, Cell Signaling Technology, Inc., Danvers, MA, USA), anti-LC3B (GTX127375, GeneTex, Inc., Irvine, CA, USA), anti-SQSTM1/p62 (GTX100685, GeneTex, Inc., Irvine, CA, USA), and anti-β-actin (GTX109639, GeneTex, Inc., Irvine, CA, USA). All primary antibodies were used at a dilution of 1:1,000, except β-actin (1:5,000). After washing, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature, and protein bands were visualized using enhanced chemiluminescence detection.

Statistical analysis

All data are expressed as mean ± standard deviation (*SD*), with n indicating the number of independent experiments. Error bars represent *SD*. Statistical analyses were conducted using GraphPad Prism software (version 9.0.0; GraphPad Software, San Diego, CA, USA). Depending on the experimental design, comparisons among multiple treatment groups were performed using one-way analysis of variance (ANOVA) followed by either Dunnett's or Tukey's post hoc test, as appropriate. For comparisons between two groups, unpaired Student's *t*-tests were applied. A P value < 0.05 was considered statistically significant.

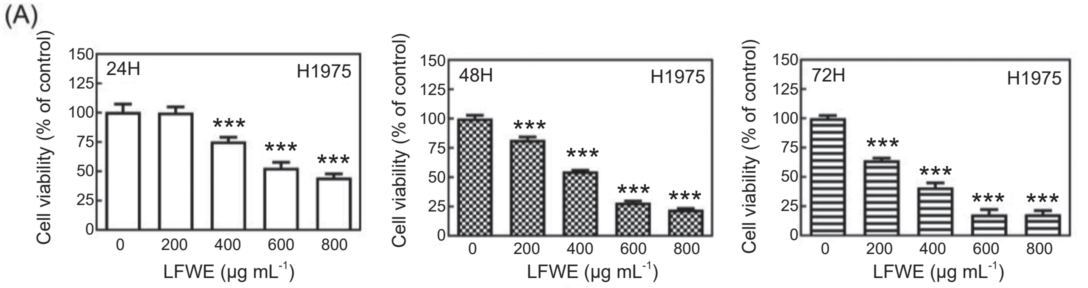
RESULTS AND DISCUSSION

LFWE inhibits H1975 cell growth and alters cellular morphology

To evaluate the cytotoxic effects of LFWE on lung cancer, we selected the human non-small cell lung carcinoma cell line H1975. This cell line harbors EGFR mutations (L858R and T790M), which are clinically associated with resistance to first- and second-generation tyrosine kinase inhibitors (TKIs), making it a relevant *in vitro* model for exploring potential alternative therapies.

MTT assays revealed that LFWE reduced H1975 cell viability in a dose- and time-dependent manner. The IC50 values decreased from 1,599.2 μg mL^{-1} at 24 h to 505.1 μg mL^{-1} at 48 h, and further to 190.0 μg mL^{-1} at 72 h (Fig. 1A and B), indicating cumulative suppression of cellular metabolic activity. Although high concentrations were required for substantial inhibition, the progressive reduction in IC_{50} suggests increased cellular sensitivity upon prolonged exposure.

Examination of cellular morphology showed marked changes following LFWE treatment. Cells shifted from an elongated, spindle-like appearance to a rounded and shrunken morphology, accompanied by a notable reduction in cell density (Fig. 1C). These alterations are consistent with cytotoxic effects. Collectively, these findings demonstrate that LFWE suppresses H1975 cell growth and affects cell morphology, prompting further investigation into potential underlying mechanisms such as cell-cycle perturbation, apoptosis, or other non-apoptotic cytotoxic processes.



(B)

H1975

IC_{50} of LFWE (μg mL^{-1})	
24 H	1599.2
48 H	505.1
72 H	190.0

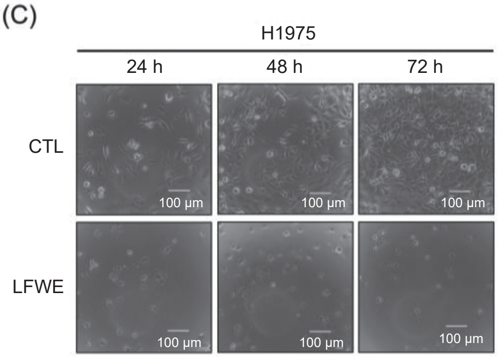


Fig. 1. Longan flower water extract (LFWE) inhibits viability of H1975 lung cancer cells. (A) H1975 cells were treated with LFWE (0–800 μg mL^{-1}) for 24, 48, and 72 h. The bar graph show the cell viability determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Data represent the mean ± *SD* (n = 3). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test comparing each treatment group with the control (0 μg mL^{-1}). Significant differences are shown ($^{***}P < 0.001$). (B) IC_{50} of LFWE in H1975 cells. (C) Morphological changes of three lung cancer cell lines after treatment with LFWE (800 μg mL^{-1}) for 24–72 h. CTL: control.

LFWE exhibits differential cytotoxicity between cancerous and normal lung cells

To evaluate the cytotoxic effects of LFWE, we examined its impact on the human non-small cell lung carcinoma cell line H1975 as well as two non-malignant lung cell lines: bronchial epithelial BEAS-2B and fibroblast WI38. These non-malignant cell types represent distinct compartments of lung tissue and provide an initial assessment of potential toxicity toward normal cells. Cells were treated with LFWE at 0–800 μg mL^{-1} for 72 h, and viability was measured using the MTT assay.

LFWE reduced H1975 viability in a dose-dependent manner, with 200 μg mL^{-1} causing moderate inhibition (66.2% viability) and higher concentrations (400–800 μg mL^{-1}) inducing substantial suppression (44.8–20.5% viability) (Fig. 2A). Importantly, BEAS-2B cells were already markedly affected at 200 μg mL^{-1} (45.1% viability), and WI38 fibroblasts displayed partial inhibition (67.4% viability), indicating that LFWE exerts cytotoxic effects on non-malignant cells even at relatively low doses. At concentrations above 600 μg mL^{-1}, H1975 cells were suppressed slightly more than either BEAS-2B or WI38, reflecting modest preferential activity toward cancer cells. For example, at 800 μg mL^{-1}, the viability of WI38 and BEAS-2B cells were significantly higher than that of H1975 cells (Fig. 2B).

Overall, these results reveal a dose- and cell type–dependent cytotoxic profile. The early sensitivity of BEAS-2B epithelial cells at 200 μg mL^{-1} and the limited differential between malignant and non-malignant cells highlight a narrow therapeutic window. These findings underscore the necessity of carefully evaluating the molecular mechanisms underlying LFWE's effects on cancer versus normal lung cells to inform its potential translational application and safety profile.

LFWE perturbs cell-cycle progression and induces a modest apoptotic response in H1975 cells

Flow cytometric analysis revealed that LFWE altered H1975 cell-cycle distribution. The G1 fraction decreased progressively with increasing dose (from ~58% to ~48%), while the S-phase fraction increased (from ~5% to ~16%), and G2/M remained relatively stable. The sub-G1 population, typically associated with apoptotic DNA fragmentation, increased slightly (from < 1% to ~2%) (Fig. 3A). These results suggest that LFWE disrupts normal cell-cycle progression, promoting premature

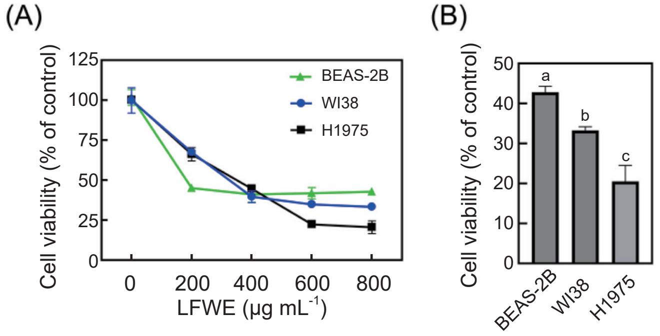


Fig. 2. Effects of longan flower water extract (LFWE) on the viability of lung cancer and normal lung cells. (A) H1975 lung adenocarcinoma cells and two normal lung cell lines (bronchial epithelial BEAS-2B and fibroblast WI38) were treated with LFWE (0–800 μg mL^{-1}) for 72 h. Cell viability was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Data represent the mean ± *SD* (*n* = 3). (B) The bar graph compares cell viability at 800 μg mL^{-1} LFWE. Data represent the mean ± *SD* (*n* = 3). Statistical significance between cell types was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test; groups not sharing the same letter are significantly different.

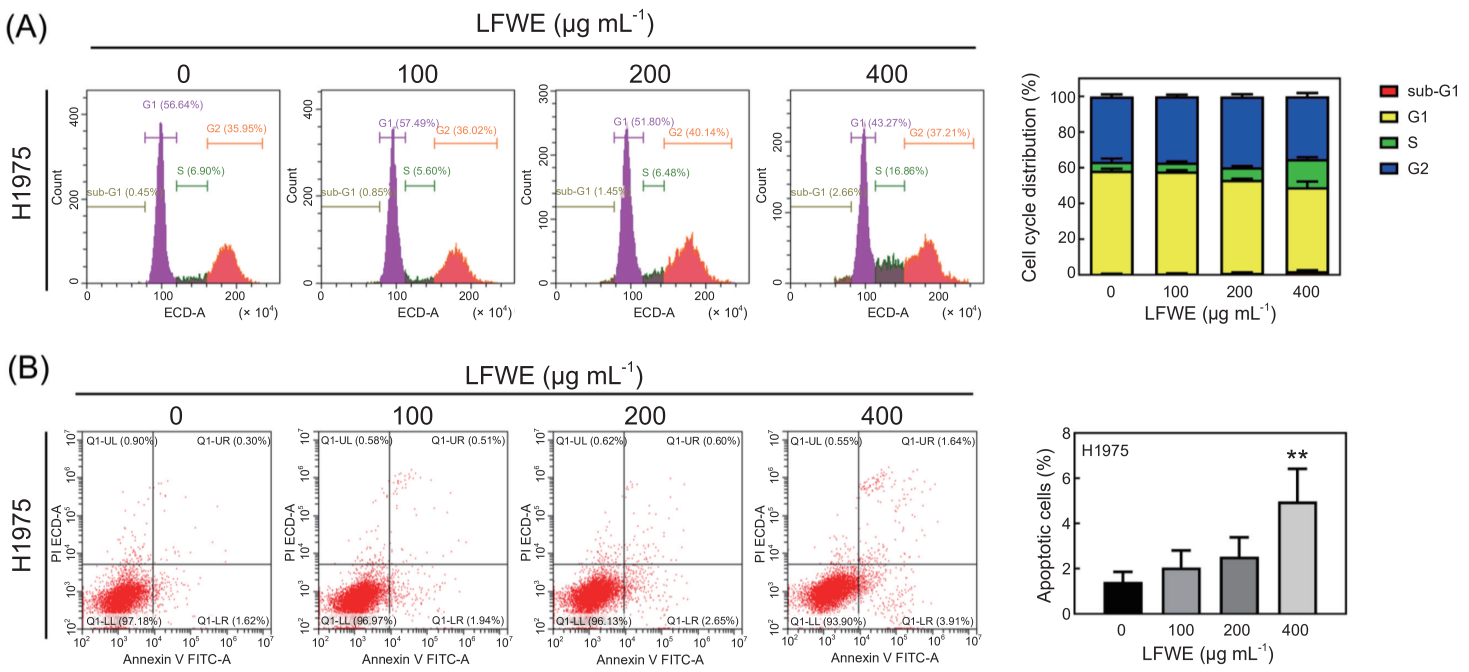


Fig. 3. Longan flower water extract (LFWE) enhances subG1 population and induces minor apoptotic responses in H1975 lung adenocarcinoma cells. (A) H1975 cells were treated with LFWE (0–400 μg mL^{-1}) for 48 h, stained with propidium iodide (PI), and analyzed for cell cycle distribution by flow cytometry. (B) H1975 cells were treated with LFWE (0–400 μg mL^{-1}) for 48 h, co-stained with Annexin V-FITC (fluorescein isothiocyanate) and PI, and analyzed for apoptosis by flow cytometry. Data represent the mean ± *SD* ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test comparing each treatment group with the control (0 μg mL^{-1}). Significant differences are shown ($^{**}P < 0.01$).

or dysregulated entry into S phase, which may compromise genomic integrity and contribute to growth suppression.

Annexin V/propidium iodide staining confirmed that LFWE induced apoptosis in a dose-dependent manner, but the effect was modest, reaching approximately 6% at 400 μg mL^{-1} (Fig. 3B). This limited apoptotic response suggests that apoptosis alone cannot fully account for the observed decrease in viability, and additional non-apoptotic cytotoxic mechanisms are likely involved.

LFWE induces autophagy-related molecular changes in H1975 cells

Autophagy is closely associated with cancer cell growth regulation (Hsin *et al.* 2011; Jung *et al.* 2020). To explore potential non-apoptotic mechanisms, we analyzed key autophagy-related proteins microtubule-associated protein 1 light chain 3 (LC3) and sequestosome 1 (SQSTM1, also called P62). LC3 exists in two forms: LC3-I, the cytosolic unlipidated form, and LC3-II, the lipidated form that associates with autophagosome membranes. The LC3-II/LC3-I ratio is commonly used to assess autophagosome formation and overall autophagic activity. P62 functions as an autophagic adaptor by binding ubiquitinated proteins and LC3-II, directing them to autophagic degradation. Under active autophagy, the LC3-II/LC3-I ratio increases, while P62 levels decrease.

Western blot analysis revealed that LFWE treatment increased the LC3-II/LC3-I ratio from 1 (control) to 2.11, 4.20, and 3.90 at 100, 200, and 400 μg mL^{-1}, respectively. Concurrently, P62 levels significantly decreased from 1 (control) to 0.43, 0.31, and 0.33 (Fig. 4). These results demonstrate a significant induction of autophagic activity by LFWE in H1975 cells, which may contribute to the observed growth suppression.

LFWE downregulates mutant EGFR through a lysosome-associated mechanism and suppresses downstream signaling

EGFR, particularly its activating mutations, is a clinically relevant therapeutic target in lung adenocarcinoma (Sharma *et al.* 2007). Conventionally, EGFR TKIs, such as erlotinib and gefitinib, suppress EGFR signaling by inhibiting kinase activity at the adenosine triphosphate (ATP)-binding site. However, H1975 cells harbor the EGFR L858R/T790M double mutation (Tang *et al.* 2008), which confers constitutive signaling and resistance to first-generation EGFR-TKIs by restoring ATP affinity and preventing effective kinase inhibition.

Given this therapeutic challenge, we investigated whether LFWE could modulate EGFR signaling in H1975 cells. We measured mutant EGFR protein levels and its downstream effectors, phosphorylated mammalian target of rapamycin (pmTOR) and phosphorylated AKT (pAKT). LFWE reduced mutant EGFR expression in a concentration-dependent manner, from 1 (control) to 0.86, 0.59, and 0.43 at 100, 200, and 400 μg mL^{-1}, respectively. pmTOR levels decreased from 1 to 0.74, 0.34, and 0.42, while pAKT decreased from 1 to 0.99, 0.86, and 0.62 across the same concentration series (Fig. 5A). At 200 and 400 μg mL^{-1}, mutant EGFR and pmTOR expression levels were significantly reduced, whereas pAKT showed a downward trend without reaching statistical significance (Fig. 5B). These results indicate that LFWE inhibits the mutant EGFR-mTOR axis, with a possible but not statistically confirmed impact on AKT signaling.

Since LFWE markedly reduced mutant EGFR protein expression, we next investigated whether this effect was associated with lysosome-mediated degradation. Treatment with the lysosomal inhibitor Bafilomycin A1 (BafA1) partially reversed the LFWE-induced decrease in EGFR levels, resulting in no significant difference between the LFWE and control groups (Fig. 6). This indicates that lysosomal activity may contribute to LFWE-mediated EGFR downregulation. Together with the observed activation of autophagic markers, the findings suggest that LFWE may promote lysosome-associated EGFR turnover, thereby attenuating oncogenic signaling in H1975 cells.

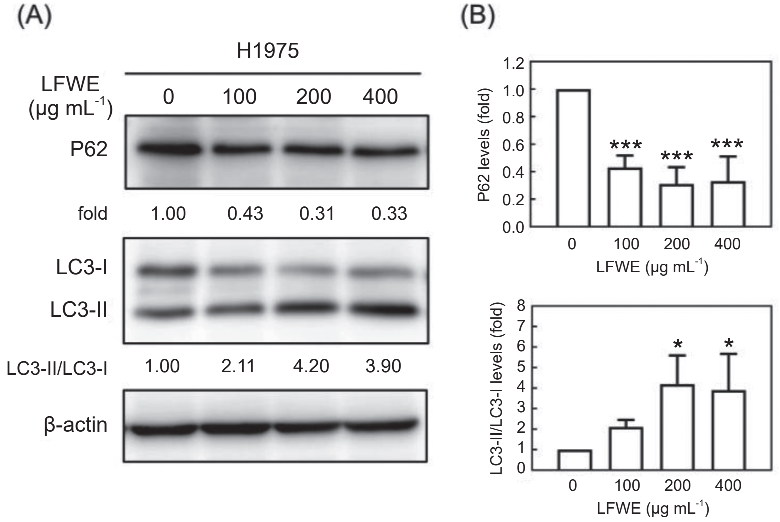


Fig. 4. Longan flower water extract (LFWE) induces autophagic response in H1975 lung adenocarcinoma cells. (A) H1975 cells were treated with LFWE (0–400 μg mL^{-1}) for 48 h, and levels of autophagy-related proteins (LC3-I/II, P62) were analyzed by Western blot. β-Actin served as a loading control. (B) The bar graphs show protein band intensities of LC3-I/II and P62, normalized to β-actin and expressed as fold change relative to 0 μg mL^{-1} (set as 1) at each LFWE concentration. Values are presented as mean ± *SD* ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test comparing each treatment group with the control (0 μg mL^{-1}). Significant differences are indicated ($^{*}P < 0.05$, $^{***}P < 0.001$).

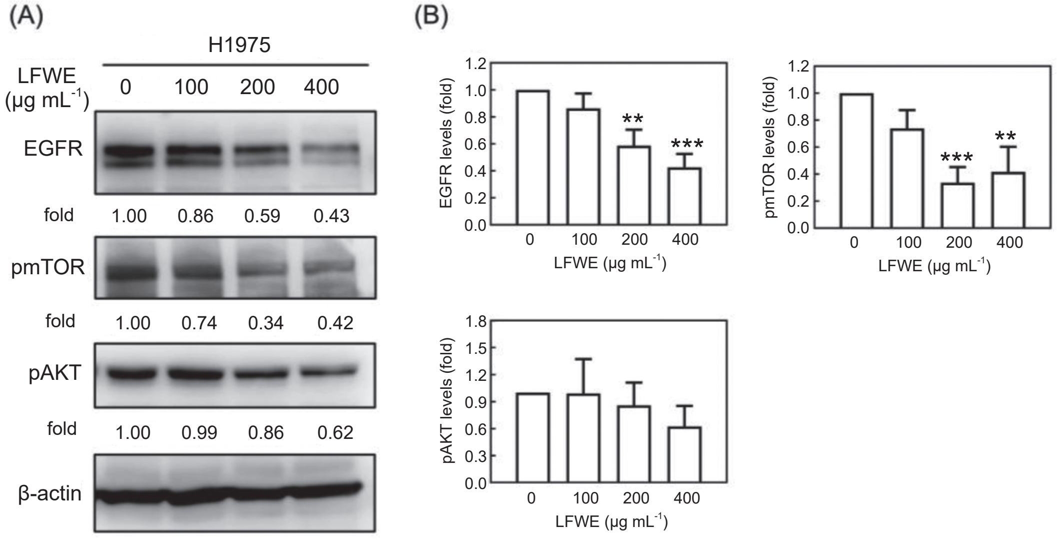


Fig. 5. Longan flower water extract (LFWE) inhibits mutant epidermal growth factor receptor (EGFR)-mediated signaling in H1975 lung adenocarcinoma cells. (A) H1975 cells were treated with LFWE (0–400 μg mL^{-1}) for 3 h, and protein levels of mutant EGFR and its downstream effectors (pmTOR, pAKT) were analyzed by Western blot. β-Actin served as a loading control. (B) The bar graphs show protein band intensities normalized to β-actin and expressed as fold change relative to 0 μg mL^{-1} (set as 1) for each protein. Values are presented as mean ± *SD* ($n = 3$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test comparing each treatment group with the control (0 μg mL^{-1}). Significant differences are indicated ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

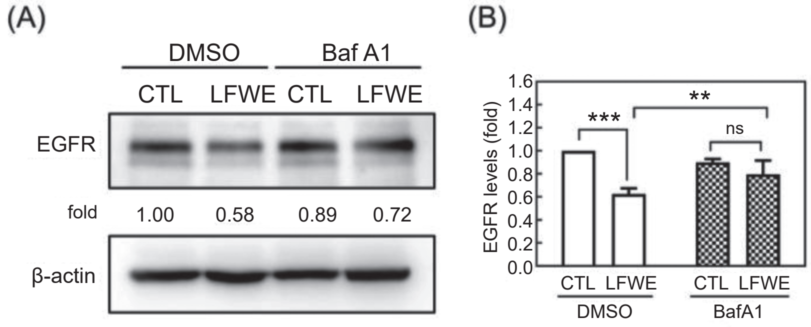


Fig. 6. Bafilomycin A1 attenuates LFWE (longan flower water extract)-induced degradation of mutant epidermal growth factor receptor (EGFR) in H1975 cells. (A) H1975 cells were pretreated with dimethyl sulfoxide (DMSO; vehicle) or Bafilomycin A1 (BafA1; 20 nM) for 30 min, followed by incubation with LFWE (200 μg mL^{-1}) for 3 h. Mutant EGFR levels were analyzed by Western blot, with β-actin serving as a loading control. (B) The bar graph shows quantified protein band intensities normalized to β-actin. Data represent mean ± *SD* ($n = 3$). Statistical significance was determined using Student's *t*-test to compare (i) control (CTL) vs. LFWE under DMSO, (ii) CTL vs. LFWE under BafA1, and (iii) LFWE (DMSO) vs. LFWE (BafA1). Significant differences are indicated (ns: non-significant, $^{**}P < 0.01$, $^{***}P < 0.001$).

CONCLUSION

LFWE exerts multiple anticancer effects in H1975 lung adenocarcinoma cells, including autophagy induction and lysosome-mediated mutant EGFR degradation, leading to inhibition of downstream mTOR and AKT signaling. However, LFWE exhibited a degree of toxicity toward non-malignant lung cells, and its apoptosis-inducing effect was modest. These limitations highlight the importance of further evaluating LFWE's safety and developing strategies to mitigate off-target effects in translational applications. Future studies should investigate whether low-dose LFWE in combination with conventional chemotherapeutics could enhance anticancer efficacy while reducing toxicity. In addition, isolating and characterizing its active components may provide safer and more potent anticancer agents. Together, these directions could broaden the translational potential of LFWE-based interventions against lung adenocarcinoma.

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龍眼花水萃物對肺腺癌細胞的抑制潛力與分子作用機制

洪子桓 [1] 邱偉綸 [2] 林志虎 [2] 曾愛容 [3] 林東毅 [4,*]

摘要

洪子桓、邱偉綸、林志虎、曾愛容、林東毅。2026。龍眼花水萃物對肺腺癌細胞的抑制潛力與分子作用機制。台灣農業研究 75(1):159–169。

肺腺癌是非小細胞肺癌中最常見的亞型，具有高發生率與低存活率。天然植物來源化合物提供了抗癌藥物開發的潛在來源。本研究探討龍眼花水萃物 (longan flower water extract; LFWE) 對 H1975 肺腺癌細胞的抑制作用及分子機制。LFWE 顯著降低 H1975 細胞存活率，並誘導明顯的形態變化。在機制方面，LFWE 激活自噬，表現為 LC3-II/LC3-I 比值增加與 P62 水平下降，並促進突變型表皮生長因子受體 (epidermal growth factor receptor; EGFR) 的溶酶體介導降解，進而抑制下游 mTOR 信號通路。然而，LFWE 對非惡性肺細胞亦有一些抑制效果，可能造成應用上的限制。進一步的安全性評估與轉化應用研究是必要的。總結來說，本研究提供初步證據，支持 LFWE 作為肺腺癌潛在治療策略的探索。

關鍵詞：龍眼花水萃物、肺腺癌、自噬、表皮生長因子受體 (EGFR)。

投稿日期：2025 年 9 月 25 日；接受日期：2025 年 11 月 5 日。
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第七十五卷　第一期　　中華民國一一五年三月三十一日

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Vol. 75 No. 1 March 31, 2026

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